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# Management of Rhizoctonia Aerial Blight of Soybean and Biology of Sclerotia of Rhizoctonia Solani Kuhn (Louisiana).

Gary Fischer Joye
*Louisiana State University and Agricultural & Mechanical College*

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Joye, Gary Fischer, Ph.D.

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# MANAGEMENT OF RHIZOCTONIA AERIAL BLIGHT OF SOYBEAN AND BIOLOGY OF SCLEROTIA OF *RHIZOCTONIA SOLANI* KUHN

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
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in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Plant Pathology
and Crop Physiology

by
Gary Fischer Joye
B.S., University of Georgia, 1980
M.S., Northeast Louisiana University, 1982
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To

MARIANNE

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ABSTRACT

Management of Rhizoctonia Aerial Blight of Soybean and Biology of Sclerotia of Rhizoctonia solani Kuhn

Rhizoctonia Aerial Blight (RAB) has caused severe damage on susceptible soybean cultivars throughout the southern soybean growing areas in Louisiana and the southeastern United States. During the 1983, 1984, and 1985 growing seasons, several greenhouse and field experiments were conducted to determine the influence of cultural practices (row spacings, plant populations, potassium fertilizer rates, and fungicide treatments) on RAB. A row spacing of 25 cm resulted in higher yields than row spacings of 50, 75, or 100 cm, regardless of disease rating. After the crop canopy had reached its maximum density, disease ratings were not significantly different between row spacings or plant populations. An interactive effect between increasing potassium rates and fungicide treatments had positive effects on yield through the reduction of RAB. There was a negative correlation between factor 1 (the correlation of the plant nutrients; P, Ca, Fe, and N considered as a single variable) on yield and a positive correlation with RAB disease ratings. Factor 4 (the correlation of the plant nutrient Mg and leaf area considered as a single variable) was positively correlated with yield and negatively correlated with RAB disease ratings.

Viability of sclerotia buried at depths of 5, 10, and 25 cm was not signigicantly different than that of sclerotia maintained on the soil surface (0 cm). During a 384 day period viability across all levels was reduced 60-70%.

During the time sclerotia were buried in soil, cells developed thickened walls and in some instances double walls. Also, intact monilioid cells were observed within cells apparently devoid of cytoplasm. Cytoplasm of sclerotia buried from 50 to 384 days contained an abundance of electron dense granules which were thought to be glycogen granules. Such modifications may be important in the formation of a protective cover and energy for the remaining viable but dormant cells within sclerotia.

# Chapter I

## Review of Literature

### Introduction

Aerial blight of soybean, caused by Rhizoctonia solani Kuhn anastomosis group 1 is a serious disease in Louisiana and other regions of the world (2,3,14,16,17,19,22,24,29,34,37). In Louisiana, annual yield losses due to Rhizoctonia Aerial blight (RAB) have been estimated to be 1-2% (23). Of the over 2.0 million acres of soybeans planted annually in Louisiana more than half are grown in areas where RAB has been observed (Figure 1).

RAB (20) was first reported on soybean cultivars in Louisiana in 1952 by Atkins and Lewis (3). In 1973 RAB was considered epidemic in Louisiana (18,24,25,26). The causal organism was described as Rhizoctonia solani Kuhn, the imperfect stage of Thanatephorus cucumeris (Frank) Donk. Atkins and Lewis reported the perfect stage on soybean in 1954 but it has not been observed since their initial report (2).

Presently, there are no fungicides available for control of RAB. Benlate 50WP (benomyl, E. I. du Pont Nemours and Company, Wilmington, Delaware) and Topsin M (thiophanate-methyl, Pennwalt Corporation, Philadelphia, Pennsylvania) are registered for suppression of RAB but have limited effectiveness (39,40,41,42). Resistant cultivars are only now being developed. In fungicide tests, compounds containing triphenyltinhydroxide (TPTH) have

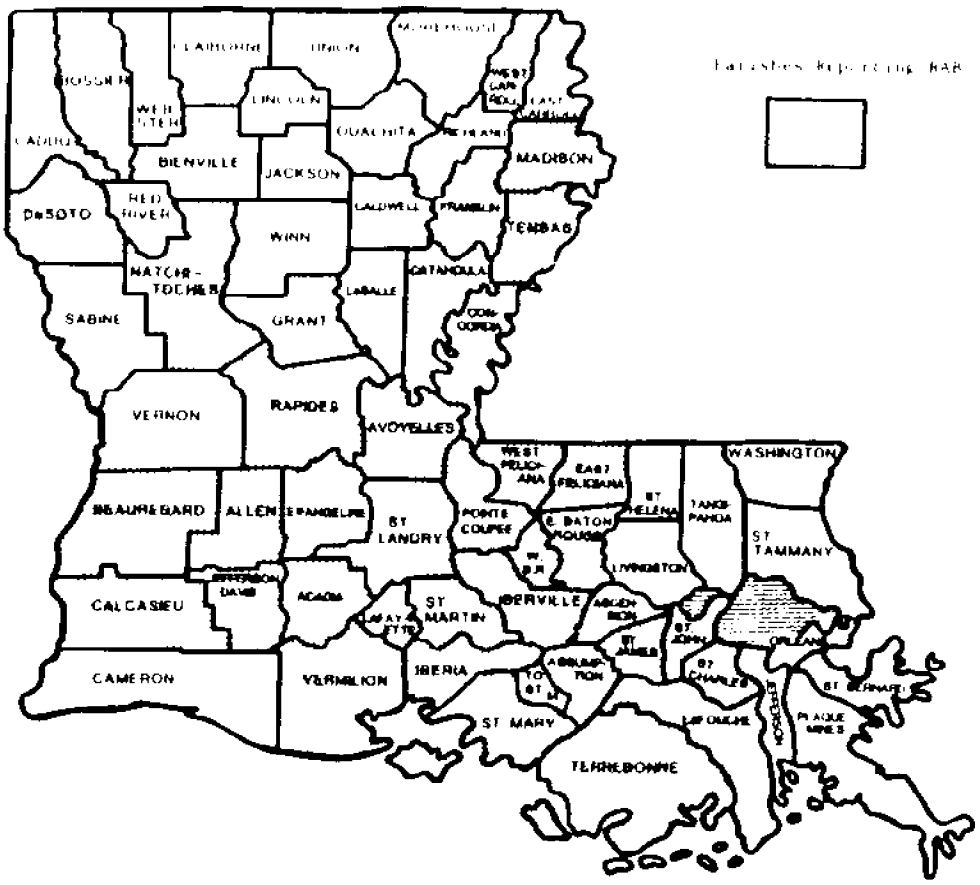


Figure 1. Distribution of Rhizoctonia Aerial Blight (RAB) in Louisiana in 1982. Data for this map was compiled from county agents reporting RAB to the Louisiana State University, Cooperative Extension Service, Baton Rouge, La.

consistently been the most effective in reducing the incidence of RAB (6,7,8,18). However, given environmental and toxicological concerns, TPTH has not been registered for use on soybeans by the United States Environmental Protection Agency. Cultivar trials have shown some differences in susceptibility but none has proven to be acceptable (6,7,8). The lack of either chemical control or cultivar resistance has changed the research focus to development of cultural practices to be used in conjunction with currently available control measures. In the past, producers have been advised to plant wide rows and utilize reduced population levels. The purpose of this practice was to allow air movement to aid drying of the crop canopy, thus lowering the amount of free moisture, which is required for the spread of RAB (42). The system currently used for the assessment of disease severity is ineffective due to the nature of the disease (10,17,22,24). This system is based on a point system ranging from 0 to 9 with 0 indicating no disease symptoms and 9 indicating 90% or more of the plants symptomatic for RAB. This system does not take into account the amount of disease present on a particular plant. The rating system proposed herein attempts to consider the amount of disease on individual plants as well as the distribution of the organism, and its disease-causing potential across an entire field.

The objectives of this research were to develop cultural practices effective in reducing the incidence and severity of RAB, to develop a disease rating system for RAB, and to investigate the influence of soil depth and time on sclerotial viability of the

pathogen causing RAB.

## The Causal Organism

Rhizoctonia solani is one of the most common plant pathogens in the world (1,9,14,19,21,24,30,37). In Louisiana, aerial types of R. solani have been reported to cause disease in bermuda grass, wheat, sugarcane, rice, sorghum, soybeans, and a number of weeds (2,3,5,14, 16,25,30). Exner (16) reported that plants belonging to the family Fabaceae had been diseased by a foliar blight caused by R. solani.

The first report of web-blight (Rhizoctonia aerial blight, RAB) to occur in the United States was in Florida on July 10, 1939 on Fordhook Lima beans (38). In 1952, Atkins and Lewis (3) reported RAB on soybean in Louisiana. The causal organism was described as Rhizoctonia microsclerotia Matz (3), later called Corticium microsclerotia (Matz) Weber (38), and finally listed as a synonym of Pellicularia filamentosa (Pat.) Rogers (31). A foliar blight of soybean caused by a Rhizoctonia sp. was reported in Louisiana in 1976 (25). The pathogen causing the disease was considered identical to the R. solani strain causing sheath blight of rice (24,25). RAB of soybean caused by R. solani, is synonomous with R. microsclerotia, the imperfect stage of the pathogen associated with the disease. The perfect state Thanatephorus cucumeris (Frank) Donk is rarely found on soybeans (2,10,24).

The symptoms of RAB have been described by several investigators (2,10,13,14,18,19,24,26). Symptoms are described as small, circular, water-soaked, light greenish-brown spots appearing on the leaf

lamina with slight reddening of the veins on the lower surface. The spots increase in size, finally becoming oblong, irregular or circular. In older spots a dark pinkish-brown margin around the light tan-brown central necrotic tissue is common. The spots may coalesce and cover major or entire portions of the leaf. Such leaves turn yellow, dry and finally drop off prematurely. Lesions on petioles and stems are light to dark-brown, oblong, 1-2 mm wide and up to 3-4 cm long. Lesions on pods are reddish-brown. Infected plants are often defoliated as younger branches near the top are blighted. The pathogen spreads mainly through contact between infected and non-infected parts of the plant by the formation of mycelial bridges. Abortion of flowers and pods is common under conditions of high humidity (> 90%) and temperatures of 32.2 to 43.3 C.

Under field conditions, a web-like mycelium with micro- and/or macrosclerotia may form on plant surfaces. Hyphae growing on host tissue are hyaline or light-brown, branched, septate, and 5-6 μm wide. Micro- and macrosclerotia are irregular or oval, white when young, changing from light to dark brown at maturity, and ranging from 2-7 mm in diameter and 1-3 mm thick.

The taxonomy and nomenclature of R. solani is based largely on the work of Duggar (15). Due to the wide and incompletely known range of variation in the species, the classification of the fungus is tentative. Parmeter and Whitney (29) have, nevertheless, published a list of characteristics they consider descriptive of R. solani.

They are:

1. Pale to dark-brown mycelia of relatively large diameter with branching near the distal septum of hyphal cells, often at nearly right angles in older hyphae.
2. Constriction of branch hyphae at the point of origin.
3. Formation of a septum in the branch at the point of origin.
4. Production of monilioid cells. Monilioid cells are short and broad with a length-to-width ratio of approximately 1-3:1 and arise as buds of preexisting cells (13).
5. Production of sclerotia of nearly uniform texture and varying in size and shape.
6. Generally, pathogenicity to a wide range of hosts, resulting in a variety of symptoms including damping off, root rot, and blighting of above ground plant tissue.
7. Possession of a prominent septal pore apparatus.
8. Possession of multinucleated cells in actively growing hyphae.

## Anastomosis Relationships Within R. solani

Rhizoctonia solani has been variously separated into at least seven distinct anastomosis groups (1,4,9,10,13,24,28,33). Anastomosis will occur among isolates within a group but not between isolates from different groups (28). Within the groups the anastomosis varied from complete fusion (with cell-killing) to cell contact without fusion. In cases where cell-killing occurred between field isolates, the fusion cell and 5-6 cells on either side became vacuolated and the cytoplasm collapsed. This suggests

genetic variability even within the groups (15). Most strains of R. solani causing foliar blight of several agronomic plants belong to anastomosis group 1 (1,5,9,16,24,28).

## Developmental Changes in Sclerotia of the RAB Fungus

Although considerable work has been done on the growth and development of mycelia and sclerotia of many isolates of Rhizoctonia solani (12,13,21,34,35,36), little has been done on the development of mycelia and sclerotia of the soybean isolate of R. solani. Exner (16) reported that the "web-blighting" fungus in Louisiana was evidently the same as the one described in 1939 by Weber (38) in Florida. Sclerotia produced by the Weber isolate were small (1-2 mm) on the host and large (3-4 mm) on artificial media and fit more closely the Matz description for Rhizoctonia dimorpha (16).

Hashiba and Mogi (21) indicated that there are changes in appearance, cytology, and buoyancy at different stages of sclerotial development. Initially the hyphal cells of the central mass of the sclerotia are larger than those of mycelium. Sclerotial development was completed within about 40 hours with maximum size after 30 hours. With increasing age of sclerotia, the cellular contents emptied from the cells of the outermost layer. The sclerotia became more buoyant with age (21).

Sclerotia are composed mainly of monilioid cells (13). On sterile media, sclerotia are composed entirely of undifferentiated hyphae, brown in color, and variable in shape. Sclerotia have been

variously described as being irregular with smooth surfaces, globose, flat and elongated, crusty, or irregularly globose with a pitted surface. Sclerotia range in size from less than 1 mm to more than 2 cm in diameter (13,21,34).

## Chemical Controls and Cultural Practices

Extensive work has been done in developing chemical controls for RAB (6,7,8,18,22). Benlate 50 WP (E. I. Du Pont Nemours and Company, Wilmington, Delaware) and Topsin M 70 WP (Pennwalt Corporation, Philadelphia, Pennsylvania) are labeled in Louisiana under section 24(c) of the Federal Insecticide, Fungicide, and Rodenticide Act for suppression of RAB. Producers are advised to spray either of these materials at a rate of .56 to 1.1 formulated kg per hectare when symptoms appear in their fields and again 2 weeks later (42).

There are no cultural practices which are effectively used in the management of RAB. Some workers (19,27) prescribe using non-host crops in rotations. In Louisiana, the effectiveness of this practice is limited because producers who rotate crops do so with rice, corn, and grain sorghum, all of which are susceptible to *R. solani* (24).

Several workers have stressed the importance of organic mulches (19,27) to reduce RAB disease but this also would be impractical for large scale monocultures of soybeans now in existence. This practice may have some value in no-till or minimum-till situations.

## Soybean Cultivar Resistance

No substantially high yielding, RAB resistant soybean cultivar has been developed for Louisiana soybean producers. However, cultivar differences in resistance within all locally grown maturity groups have been observed (6,7,8). Based on results of cultivar evaluations, moderately resistant cultivars are recommended for planting in areas with RAB. Cultivars in maturity group V that are considered to have moderate resistance to RAB include: Asgrow 5474, Bedford, Deltapine 105, Deltapine 345, Forest, TerraVig 505, and Wilstar 550. In maturity group VI cultivars considered moderately resistant include: Centennial, Coker 156, Deltapine 506, Hartz 6383, Ring Around RA680 and Tracy M. In maturity group VII moderately resistant cultivars include: Bragg, Braxton, Bossier, Hartz 7126, Wright and Coker 368 (11).

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# Chapter II

## The Effects of Rowspacing, Plant Population, and Potassium Levels on Rhizoctonia Aerial Blight of Soybean

### Introduction

The ineffectiveness of foliar fungicides and the lack of identified cultivar resistance for Rhizoctonia Aerial Blight (RAB) of soybean has prompted researchers to focus attention on cultural practices for management of RAB. Presently, there are no available fungicides which can be applied economically to maintain satisfactory RAB disease control under severe disease pressure. Benlate 50 WP (benomyl, E. I. du Pont Nemours and Company. Wilmington, Delaware) and Topsin M 70 WP (thiophanate-methyl, Pennwalt Corporation, Philadelphia, Pennsylvania) are registered for foliar application at the rate of .56 to 1.1 formulated kg per hectare at the first appearance of RAB symptoms and for a second application within 2 weeks (3,30,31,32,33). Generally, soybeans are planted in Louisiana on hipped rows with a 50 to 75 cm spacing. Producers with a history of RAB in their fields have been advised to plant wide rows (50 cm or more) and low plant populations (21-28 plants/meter row) (33). Rhizoctonia solani sclerotia, the primary inoculum for RAB, require 24 to 48 hours of continuous free moisture at 25 C (17,25) for germination. Mycelia of the fungus may grow up the stem of the plants or the sclerotia and/or mycelial fragments may be splashed by rain onto the foliage (2,12,17). The

pathogen may attack soybean plants at all stages of development through maturity (2,12,17). The role of balanced plant nutrition and especially potassium in plant disease resistance has been well documented (1,4,8,10,15,18,20,21,23,24,27).

## Materials and Methods

### Field Studies

Several field experiments were conducted during the 1983, 1984 and 1985 growing seasons. A row spacing and plant population study was conducted at the Louisiana State University Burden Research Plantation in Baton Rouge, La. in 1983 and 1984 in a field with a history of RAB. The soybean cultivar 'Davis' was used in these field studies. Treatments consisted of planting soybean in three row spacings (25, 50, and 100 cm) and one broadcast treatment. All planting treatments were split by 2 plant populations within the rows (designated high and low). High plant populations for 100 cm row spacing ranged from 33-39 plants per meter row, while low populations ranged from 26-29 plants per meter row. High plant populations for 50 cm row spacing ranged from 26-33 plants per meter row, while low populations ranged from 18-23 plants per meter row. High plant populations for the 25 cm row spacing ranged from 18-26 plants per meter row, while low populations ranged from 10-13 plants per meter row. High plant populations for the broadcast plantings ranged from 105-126 plants per square meter, while low populations ranged from 63-84 plants per square meter. There were 8

rows in the 100 cm row space plots, 16 rows in the 50 cm row space plots, and 30 rows in the 25 cm row space plots. All treatments were replicated four times. Plots were 9 m x 4 m with 2 m alleys. All treatments were arranged within a completely randomized block design. Disease ratings were taken 7 times between July 20 and September 9 in 1983, and 3 times in 1984 between July 27 and August 1, using a system described elsewhere in this text (see Chapter 2 page 18).

Weeds and insects were controlled by spraying appropriate herbicides and insecticides. Pre-emergence herbicides included: Lasso, (Monsanto, Agricultural Division, St. Louis, Missouri) at a rate of 2.24 kg ai/ha + Sencor 75 DF, (Mobay, Chemagro, Division of Baychem Corporation, Kansas City, Missouri) at a rate of 1.23 kg ai/ha. The post-emergence herbicide used to kill grass weeds was Fusilade 4E (ICI Americas, Inc., Agricultural Chemical Division, Wilmington, Delaware) at a rate of .62 kg ai/ha. Lepidopteran larvae were monitored and if economic threshold levels were met Ambush 2E (ICI, Americas, Inc., Agricultural Chemical Division, Wilmington, Delaware) was sprayed on the plots using a Solo backpack model 425 (Solo Kleinmotoren Gmbh, D-7032 Sindelfinger 6, West Germany) at a rate of .07 kg ai/ha. Plots were sprayed in 1984 on August 3. No insecticide was applied in 1983 or 1985. The middle two rows of each plot were harvested with a Hege 125B research combine (Hans-Ulrich Hege, Saatzuchtmaschinen, Hohebuch, D-7112 Waldenburg/ Wurtt, West Germany). For the broadcast planting, one combine header width (132 cm) was harvested. Seed moisture was

determined with a Burrows Digital Moisture Computer, Model 700 (Burrows Equipment Company, Evanston, Illinois). All harvested weights were adjusted to 13.0 % moisture by the following equation:

$$A(100-B)/87=C$$

where A is the actual sample weight, B is the percent moisture of the sample, 100-B is the actual dry weight, and 87 is a constant for percent dry weight at 13.0% moisture content, and C is the adjusted weight. The test area in 1983 and 1984 was planted on June 1 and harvested on November 10 and 7 respectively.

In 1985, the field studies were conducted at Perry, Louisiana in Vermilion Parish where RAB had been severe in previous years. The field studies included combinations of three rowspacings (25, 50, and 75 cm) two plant populations (high and low) within the row spacings (for 25 cm row spacing, high plant population = 18-26 plants per meter row, low plant population = 10-13 plants per meter row; for 50 cm rowspacing, high plant population = 26-33 plants per meter row, low plant population = 18-23 plants per meter row; and for 75 cm row spacing, high plant population = 33-39 plants per meter row, low plant population = 26-29 plants per meter row), fungicide treatments (with and without), and three potash levels (0, 67.3 and 268 kg K per ha). The soybean cultivar 'Davis' was used in this study. All treatments were replicated four times. In 1985, there were 12 rows in each 75 cm row space plot, 18 rows in each 50 cm row space plot, and 36 rows in each 25 cm row space plot. The experimental design was a 3 x 2 x 2 x 3 factorial. Disease ratings were made monthly throughout the season after the

first symptoms of the disease appeared. Fungicide (Mobay NTN19701 75 WP, Monceren, Mobay Chemical Company, Agricultural Division, Kansas City, Missouri) was applied at a rate of 279g ai/ha on July 24 and August 12, 1985 to appropriate plots with a Kubota L245H Research tractor (Kubota Tractor Corporation, Compton, California) equipped with a 4.5 meter side delivery, compressed air foliar spray boom. The potassium was applied as KCl using a Cyclone Seeder model 20A (Cyclone Seeder Co., Inc., Urbana, Indiana) to appropriate plots at planting. Tissue analysis consisted of subsampling leaf blades from the fourth row from the east side of each plot 2 m from the north end. A sufficient amount of foliage was collected from each plot to obtain 6 grams of dry leaf material needed for nutrient analysis. Total nutrient analysis of the plant tissue was determined for the following elements through standard methods (16) by the LSU Feed and Fertilizer Laboratory. The percentages of nitrogen (16,22), phosphorus (35), potassium (16,19), calcium (14), magnesium (14), and parts per million of manganese (14), zinc (14), iron (14), and sulfur (29) were determined. Percentages of acid detergent fiber and lignin (36) were determined by this author. Samples were taken at R2 growth stage (11). Leaf area indices were determined at R1 growth stage (11) by subsampling 1 m row from the middle row, 4 m from the north end of each plot. Collected samples were placed in plastic bags and labeled. The leaves were stripped from the stem and total leaf area of the sample was determined with a Decagon Delta-T Leaf Area Meter

(Decagon Devices, Inc., Pullman, Washington). The leaf area index was calculated as unit leaf area per unit ground area. At maturity, the middle two rows of each plot were hand-harvested and threshed with a Hege 125B research combine. All harvested weights were adjusted to 13.0% by the previously described equation. Yield was reported in kg/ha. Weeds and insects were controlled with the same chemicals and rates used in 1983 and 1984. The test area was planted on June 1 and harvested on November 6, 1985.

## The Disease Rating System

The disease rating system developed in this study took into account the intensity of the disease on individual plants as well as the severity of the disease within a plot. The disease rating was calculated as the product of the intensity and the severity values of the disease within a field. Intensity and severity ratings were between 1 and 5, with 1 being the lowest intensity or severity and 5 being the highest rating (Figure 2). The lowest overall disease rating was 1 and the highest 25 (Table 1).

To determine if the disease rating system was correlated with yield, a greenhouse study was conducted. Four growth chambers made of 5 x 5 x 244 cm pine studs and polyurethane were fixed on greenhouse benches that had been filled to a depth of 30 cm with sterile growth media consisting of one part each of soil, sand, and peatmoss. The chambers were split to form 8 smaller chambers each measuring 60 x 244 x 122 cm. Free moisture was maintained by

placing a Hankscraft model 240 cool vaporizer (Gerber Products Corporation, Reedsburg, Wisconsin.) in each chamber. The soybean cultivar 'Davis' was used for this study. Seed were planted in rows 60 cm long with 25 cm spacing between rows. Each row was thinned to 20 apparently healthy plants. Inoculum of R. solani (Isolate 465 anastomosis group 1, donated by E. E. Bulter, Department of Plant Pathology, University of California, Berkeley) was grown on sterilized rice hulls in eight 3 liter flasks. After the plants had grown to the V4 growth stage (11), the inoculum (454 grams of rice hulls with mycelia) was placed at the soil line around the stems to allow the fungus to grow up the stem. Water was applied over the top of the foliage to simulate rainfall and provide a mechanism for splashing of inoculum. After early foliar symptoms appeared, the fungicide Mobay NTN19701 was applied at the rate of 0, 0.3, 0.62, and 1.23 g ai/1500 sq cm of ground area with a 350 ml Spray Pal All Purpose Sprayer model S-67 (Delta Industries, Philadelphia, Pennsylvania). Fungicide spray was applied to the point of runoff. Treatments were arranged in a completely randomized block design. Disease ratings were made on August 14, August 23 and September 9. Seeds were harvested by hand, weighed, and counted.

## Statistical Analysis

Data from the disease ratings analyses were subjected to analysis of variance procedures and regression analyses was made on yield and disease ratings by fungicide treatments. Data from the row

spacing and plant population analyses were subjected to analysis of variance procedures. The Duncan's New Multiple Range Test was performed on yield. Regression analysis was performed on the disease ratings for row spacing and over time. Data from the 1985 field test were subjected to analysis of variance and multivariate analysis of variance procedures. Factor and regression analyses were performed on all main effects, on all significant interactions and on the effects of nutrients on yield and disease ratings. Factor analysis was used in this analysis to efficiently use the data needed to determine those variables (plant nutrients) which were correlated. All analyses were made using the Statistical Analysis System (SAS) on the Louisiana State University mainframe computer system.

Tables and Figures of analysis of data appear in Appendix 1 following the literature cited.

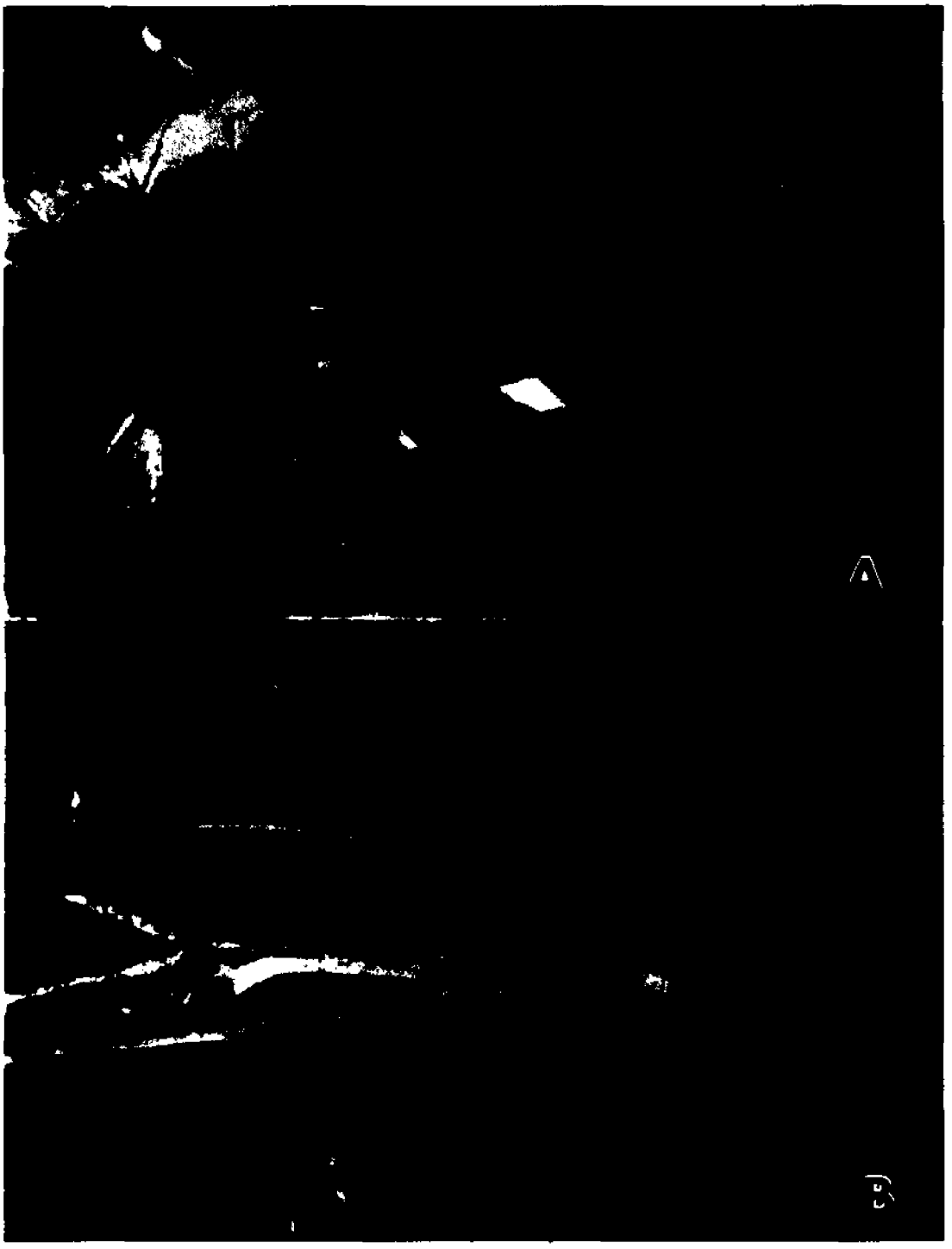


Figure 2. Symptoms of Rhizoctonia Aerial Blight on 'Davis' soybean with a severity rating of 1 (A) and 4 (B).

Table 1. Rhizoctonia Aerial Blight Rating Disease System*

| Severity | Distribution (100 plants) |
|---|---|
| 1 = Initial small water-soaked lesion near leaf bases, becoming necrotic upon drying. Less than 1/2 leaf area diseased. | 1 = 1-20 infected plants |
| | 2 = 21-40 |
| | 3 = 41-60 |
| 2 = Water soaked & necrotic lesions over more than 1/2 leaf area. | 4 - 61-80 |
| | 5 = 81-100 |
| 3 = As in 2 with additional mycelial webbing to leaves of neighboring plants. | |
| 4 = Severe leaf necrosis, webbing over most plant parts, stem necrosis, pod abortion, sclerotia present. | |
| 5 = Plants dying or dead | |

* Severity x Distribution = Disease Rating

## Results and Discussion

### The Disease Rating System

In the greenhouse, yield components (seed number, seed weight in grams) and disease ratings were significantly influenced by increasing fungicide rates (Table 2). The number of seeds and grams of seed were inversely related to disease ratings (Figure 3), suggesting that increased yield may be obtained when RAB is lowered. Because of the significant correlation between yield response and disease ratings and the rapid evaluation of RAB, this system was thought to be adequate and effective for the evaluation of RAB in small field plots.

### 1983 Field Study

Average germination of all plots was 73%. Environmental conditions were favorable following planting, contributing to the development of early season symptoms of RAB. Symptoms of RAB were observed on soybean foliage after the V5 growth stage (11) on July 5. The results of the analysis of variance procedure indicated that yield was significantly influenced by row spacing (Table 3). Plant density had no effect on yield. Yield significantly increased as row spacing narrowed (Figure 4). Higher yields were recorded in the 25 cm row spacing than for wider spacings (100 or 50 cm). The lowest yields, however, were in the broadcast treatment. This was no doubt due to severe lodging which led to an inefficient harvest.

These results were consistent with other reports on the effects of row spacing on yield. In the Midwest, soybean yields have been higher when they are planted on rows spaced 50 cm or less when compared to wider row spacings (90 cm or greater) (34). In the southern United States, soybean yields have been inconsistent when planted in narrow rows. Carter and Boerma (7) have indicated that yields are greater on narrow rows, while Hartwig (13) reported no yield enhancement from narrow rows over wider rows. In Louisiana, Boquet et al.(5,6) and Williams and Noor (34) indicated that soybean cultivars grown on 50 cm rows resulted in higher yields than 100 cm rows, but found that yields were inconsistent on 25 cm rows and depended on the variety.

Disease ratings were made seven times between July 20 and September 9. Analysis of variance procedures showed that the disease ratings were significantly effected by row space and time of season (Table 4). Disease ratings became lower as row width increased (Figure 5). The spread of RAB was also continuous until plant senescence (Figure 6). These results are, however, contrary to control recommendations presently in the literaure (26). Contemporary recommendations suggest avoiding planting densities and row spacings that promote poor aeration of foliage, albeit there are no published results supporting those recommendations for the control of RAB.

1984 Field Study

Average germination for all treatments was 76%. Environmental conditions were favorable following planting, which aided in the development of early season symptoms of RAB, as in 1983. Symptoms were observed on soybean foliage after most plants were at the V5 growth stage (11). Disease development was limited in 1984 due to drier enviromental conditions. The analysis of variance procedure indicated that yield was significantly influenced by row spacing (Table 5). Plant population had no effect on yield. Yield increased as row spacing increased from the broadcast planting to the 100 cm row spacing (Figure 7). This was inconsistent with results from 1983 but was supported by previous reports that yields vary in the narrower row spacings (34). In a study to determine the interacting effects of certain insect pests and RAB on 'Davis' soybean (which included row spacings of 25, 50 and 75 cm) yields were higher in the 25 cm rows than in the 50 or 75 cm rows (Yanes, J., and G.F. Joye, 1984, unpublished data).

The analysis of variance precedure (Table 6) showed that row spacing and time of growing season significantly influenced disease ratings. Plant population had no effect on disease ratings. The disease rating decreased as row spacing became wider (Figure 8). These results were consistent with present recommendations to avoid conditions that favor poor aeration, but were contrary to the results of 1983. This may have been due to a much drier growing season in 1984 than that of 1983, or because early season flooding in 1984 immediately following planting caused a delay in maturity

in some of the narrow row plots. As in 1983, disease ratings were higher as the season progressed (Figure 9).

1985 Field Study

Average germination was 77% for all plots. Symptoms of RAB were observed in most plots on June 22 as environmental conditions became favorable during this time. Soybean plants had reached the V8 growth stage (11). Symptoms were in early stages of development with an average disease rating of 2 for all treatments. The pathogen was allowed to continue growth throughout the crop canopy until July 24 when the foliar fungicide was applied. Disease ratings at this time averaged 3 for all plots. During the latter part of the growing season, environmental conditions became severe as the hurricane season reached its peak with 32.8 cm of precipitation recorded in August. This increase in moisture greatly enhanced the pathogen's growth thoughout the crop canopy. Excessive winds also caused some damage and lodging to the crop. Prior to harvest an estimated 20% of the pods had dropped. The dropped pods were not recovered.

Results of the analysis of variance procedure showed that yield was significantly influenced by row spacing (Table 7). Yield was higher in the 25 cm row spacing than either the 50 or 75 cm row spacing (Figure 10). These results were similar to results from 1983 but not with results from 1984.

RAB disease ratings increased until plant senescence. As in 1983 and 1984 disease ratings were influenced by row space and were also

affected by fungicide applications (Table 8). Disease ratings tended to decrease as row spacing increased from 25 to 75 cm and were further reduced with the additions of foliar fungicide (Figure 11). An interaction between fungicide and potassium also had a significant impact on disease ratings (Figure 12). Disease ratings were shown to decrease as the level of potassium increased if foliar fungicide was also applied. However, disease ratings increased with increasing levels of potassium when no fungicide was applied. The increase in disease ratings with increasing potassium rate is not fully understood. However, high levels of potassium have been known to increase the disease severity in other crops (28). Prior to this experiment, analysis of soil samples had indicated high levels of potassium were available in the soil (1818 kg/ha). Additional application of potassium may have had no impact or may have been antagonistic to RAB under these conditions.

From the factor analyis procedure (9) (see Appendix 2 for program), data from the tissue analyses showed that several nutrients were correlated and had significant effects on both yield (harvest weight) and disease ratings. The nutrient combinations which were correlated make up a single factor. These factors can be considered as one variable. Five factors were listed from results of analysis of data (Table 9). In addition to the influence of row space and the potassium x fungicide interaction, factors 1 and 4 were shown to have a significant effect on yield (Table 10) and disease ratings (Table 11). From regression analysis, decreasing yield was correlated with increasing scores of factor 1 (Figure 13)

and increasing yield was correlated with increasing scores of factor 4 (Figure 14). Even though these effects were significant there was a considerable amount of experimental variation with $R^2$ of only .33. The effects of factors 1 and 4 on disease ratings were opposite those on yield. As scores of factor 1 increased, disease ratings increased (Figure 15) and as scores of factors 4 increased, disease ratings decreased (Figure 16). Since nitrogen was an important element of factor 1, this is considered to be a reasonable relationship as excessive nitrogen has been known to increase disease incidence in other crops. The effect of factor 4 (composed of leaf area indices and magnesium) also was thought to be reasonable. As disease ratings decrease, leaf area would increase and thus magnesium would increase since this element is the central component to the chlorophyll molecule. However, again there was considerable variation in this relationship with an $R^2$ of only .25. The effects of nutrients on RAB have not been extensively examined. Results presented here suggest that more knowledge may be gained through continued research emphasis in this area.

## Conclusions

The following conclusions are drawn:

1. Yields were significantly higher in the narrow row spacing (except in 1984) regardless of disease rating.
2. Disease rating was significantly influenced by row spacing with less disease occurring as row spacing became wider.
3. The interaction between increasing levels of potassium and

fungicide application had a significant effect on decreasing disease rating.

3. Factor 1 (P, Ca, Fe, and N) was negatively correlated with increasing yield.

4. Factor 4 (Mg and leaf area index) was positively correlated to increasing yield.

5. As the scores of factor 1 increased, the level of disease also increased based on the disease rating system.

6. As the scores of factor 4 increased, the level of disease decreased based on the disease rating system.

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# APPENDIX 1

# OF

# TABLES AND FIGURES

Table 2. Influence of four fungicide rates (0, 0.3, 0.62, and 1.23 g ai / 1500 sq cm) on yield (number of seed and seed weight) and Rhizoctonia Aerial Blight disease ratings.

| Dependent Variable | Source | df* | SS | MS | F | Prob. |
|---|---|---|---|---|---|---|
| Number of Seed | | | | | | |
| | Block | 7 | 6239.0 | 891.29 | 1.61 | .1871 |
| | Fungicide | 3 | 9188.75 | 3062.92 | 5.53 | .0058 |
| | Error | 21 | 11622.25 | 553.44 | | |
| | Contrast | | | | | |
| | Linear | 1 | 9188.63 | 9188.63 | 16.60 | .0005 |
| Seed Weight | | | | | | |
| | Block | 7 | 184.77 | 26.40 | 2.74 | .0347 |
| | Fungicide | 3 | 140.53 | 46.84 | 4.86 | .0101 |
| | Error | 21 | 202.41 | 9.64 | | |
| | Contrast | | | | | |
| | Linear | 1 | 132.21 | 132.21 | 13.72 | .0013 |
| Disease Rating | | | | | | |
| | Block | 7 | 136.22 | 19.46 | 1.04 | .4326 |
| | Fungicide | 3 | 329.10 | 109.70 | 5.87 | .0045 |
| | Error | 21 | 392.16 | 18.67 | | |
| | Contrast | | | | | |
| | Linear | 1 | 303.47 | 303.47 | 16.25 | .0006 |

* df = degrees of freedom, SS = Sum of Squares, MS = Mean squares

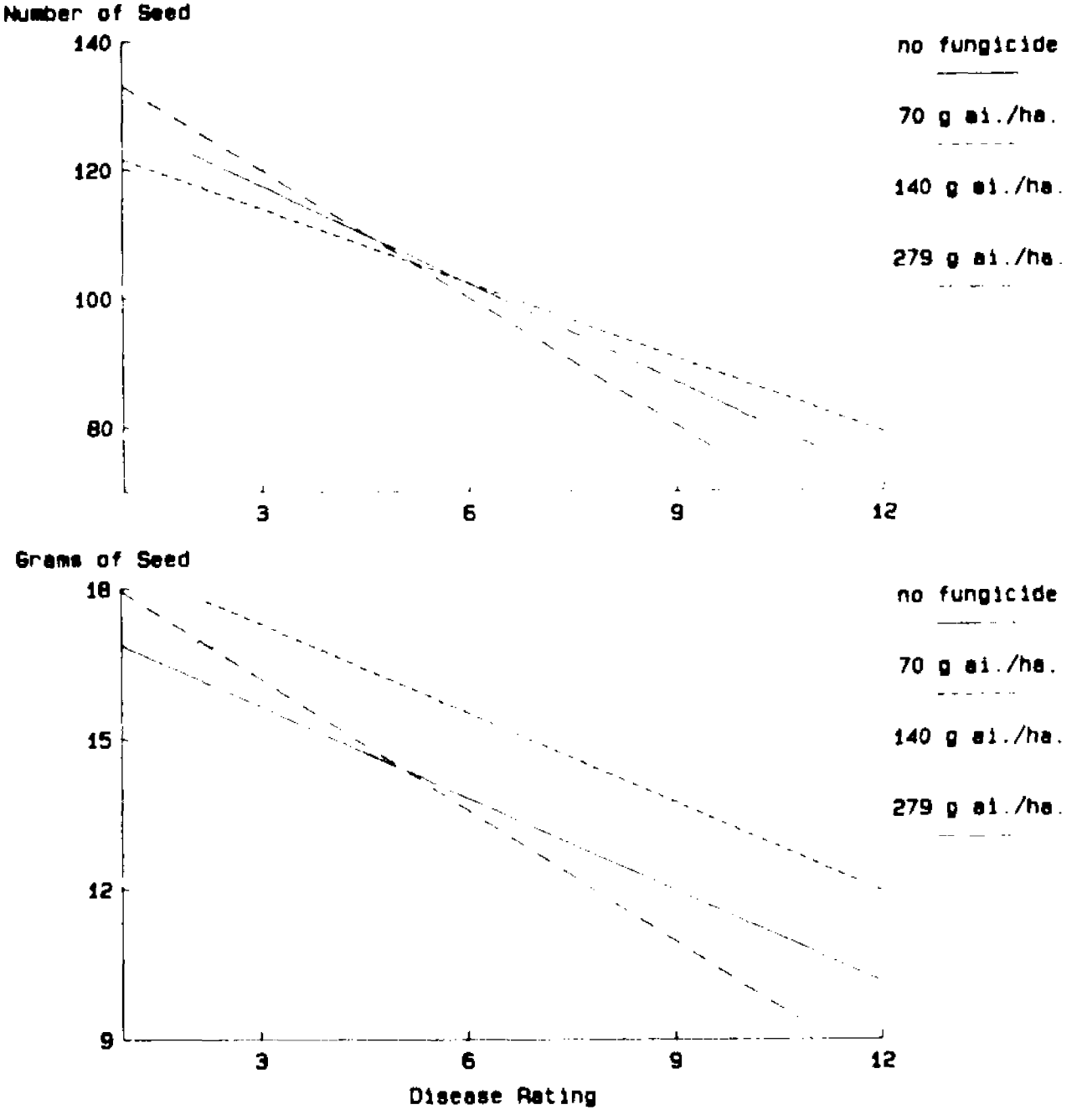


Figure 3. Relationship between yield (number of seed and seed weight) and disease ratings by fungicide rates. $R^2$ = .72, .50, .56, and .71 for 0, 70, 140, and 279 g ai. of fungicide, respectively, for each line for number of seed. $R^2$ = .65, .24, .28, and .49 for 0, 70, 140, and 279 g ai/ha of fungicide, respectively, for each line for seed weight. The fungicide used in this experiment was Mobay NTN19701 (Monceren).

Table 3. Influence of row spacing and plant population on yield of 'Davis' soybeans grown in a field infested with the Rhizoctonia Aerial Blight pathogen Rhizoctonia solani, Anastomosis Group 1. Burden Research Plantation, Baton Rouge, La., 1983.

| Source* | df | Sum of Squares | Mean Squares | F | Prob. |
|---|---|---|---|---|---|
| B | 3 | 205.626 | 68.542 | 2.50 | .1089 |
| R | 3 | 1926.221 | 642.074 | 23.44 | .0001 |
| P | 1 | 10.351 | 10.351 | 0.38 | .5502 |
| RP | 3 | 266.886 | 88.962 | 3.25 | .0600 |
| Error | 21 | 328.693 | 27.391 | | |

* B = Block, R = Rowspace, P = Plant Population

Table 4. Influence of row spacing, plant population and date on Rhizoctonia Aerial Blight on 'Davis' soybean. Burden Research Plantation, Baton Rouge, La. 1983.

| Source* | df | Sum of Squares | Mean Squares | F | Prob. |
|---|---|---|---|---|---|
| B | 3 | 259.620 | 86.400 | 4.90 | .0027 |
| R | 3 | 348.547 | 116.183 | 6.45 | .0004 |
| P | 1 | 63.377 | 63.377 | 3.22 | .0744 |
| RP | 3 | 72.234 | 24.078 | 1.27 | .2855 |
| Da | 6 | 623.261 | 103.877 | 5.82 | .0001 |
| RPDa | 42 | 720.241 | 17.149 | 0.96 | .5470 |
| Error | 165 | 2947.921 | 17.866 | | |

* B = Block, R = Row space, P = Plant Population, Da = Date

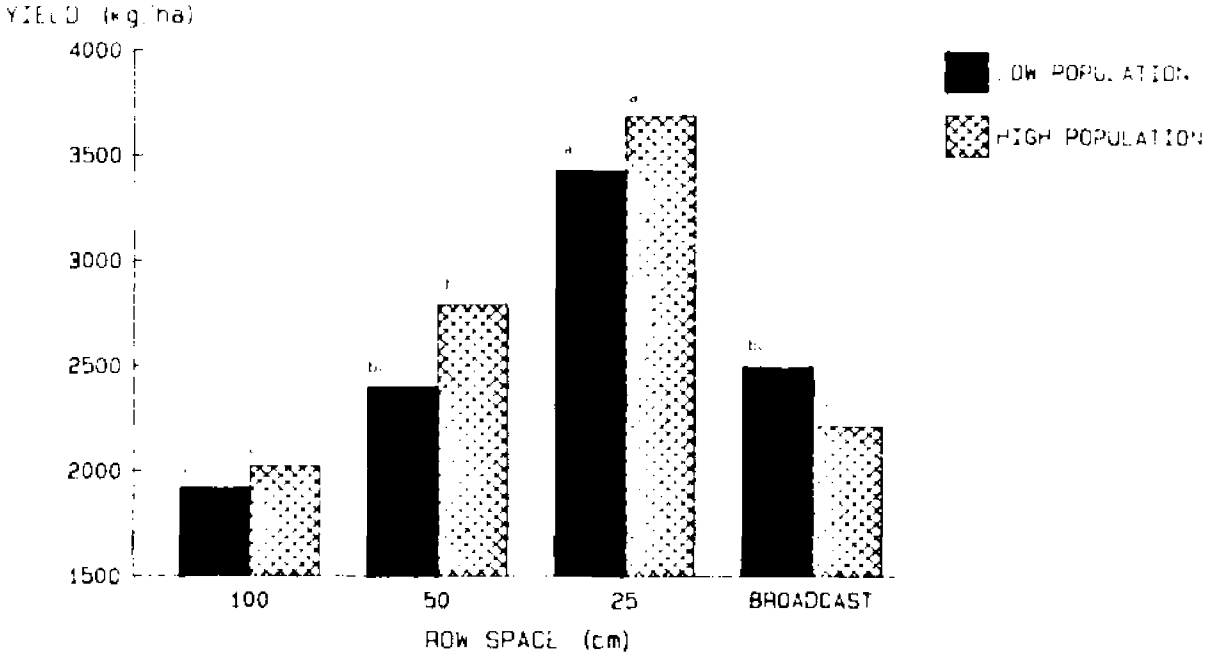


Figure 4. Yield response of 'Davis' soybean on four row spacings (broadcast, 25, 50, and 100 cm) split by plant population (high and low) in a field infested with the Rhizoctonia Aerial Blight pathogen Rhizoctonia solani AG-1. Duncan's New Multiple Range Test ($P < 0.05$). Bars with the same letter are not significantly different. Burden Research Plantation, Baton Rouge, La., 1983.

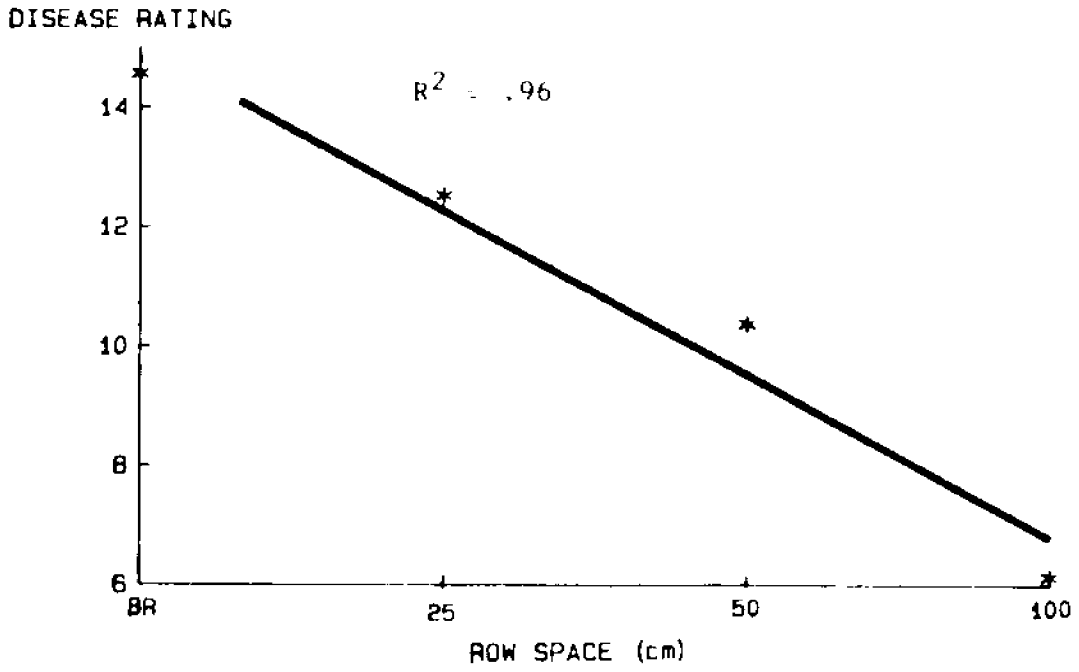


Figure 5. Relationship between Rhizoctonia Aerial Blight disease ratings on 'Davis' soybean and four row spacings (broadcast, 25, 50, and 100 cm). Burden Research Plantation, Baton Rouge, La., 1983.

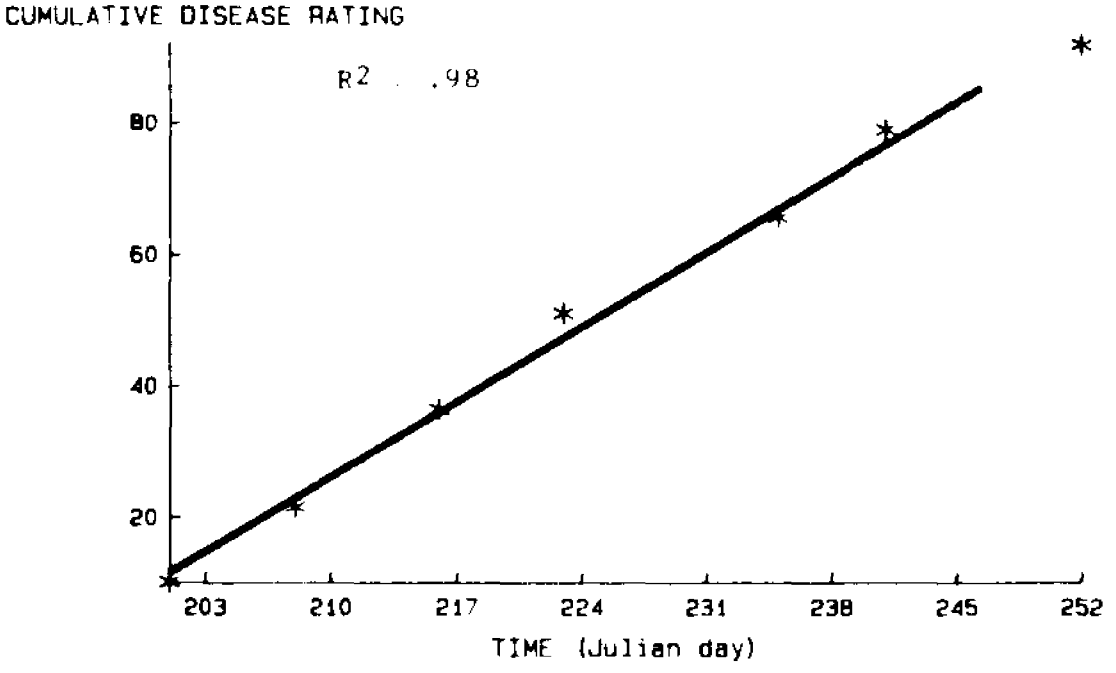


Figure 6. Relationship between Rhizoctonia Aerial Blight disease ratings on 'Davis' soybean and time of season. Burden Research Plantation, Baton Rouge, La., 1983.

Table 5. Influence of row spacing and plant population on yield of 'Davis' soybean grown in a field infested with the Rhizoctonia Aerial Blight pathogen, Rhizoctonia solani AG-1. Burden Research Plantation, Baton Rouge, La. 1984.

| Source* | df | Sum of Squares | Mean Squares | F | Prob. |
|---|---|---|---|---|---|
| B | 3 | 20.606 | 6.868 | 0.24 | .8709 |
| R | 3 | 449.868 | 149.950 | 5.13 | .0081 |
| P | 1 | 10.238 | 10.238 | 0.35 | .5602 |
| RP | 3 | 256.411 | 85.470 | 2.93 | .0576 |
| Error | 21 | 613.537 | 29.210 | | |

* B = Block, R = Row space, P = Plant Population

Table 6. Influence of row space, plant population, and date on Rhizoctonia Aerial Blight disease ratings on 'Davis' soybean. Burden Research Plantation, Baton Rouge, La., 1984.

| Source* | df | Sum of Squares | Mean Squares | F | Prob. |
|---|---|---|---|---|---|
| B | 3 | 47.614 | 15.871 | 1.74 | .1681 |
| R | 3 | 153.031 | 51.010 | 5.60 | .0091 |
| BR | 9 | 124.344 | 13.816 | 1.52 | .1631 |
| P | 1 | 8.760 | 8.760 | 0.96 | .3308 |
| RP | 3 | 1.781 | 0.594 | 0.07 | .9781 |
| Da | 2 | 1582.937 | 791.468 | 86.85 | .0001 |
| RDa | 6 | 20.562 | 3.472 | 0.38 | .8914 |
| PDa | 2 | 3.646 | 1.823 | 0.20 | .8192 |
| RPDa | 6 | 37.688 | 6.281 | 0.69 | .6590 |
| Error | 60 | 546.792 | 9.113 | | |

*B = Block, R = Row Space, P = Plant Population, Da = Date

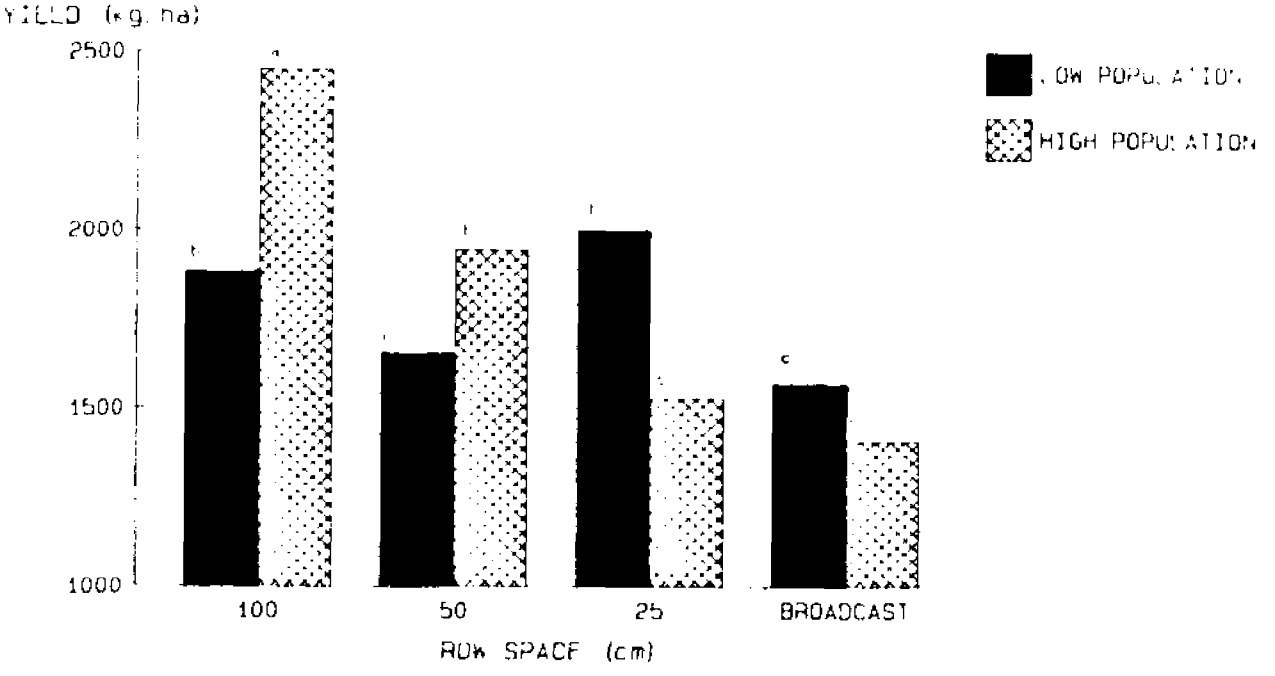


Figure 7. Yield response of 'Davis' soybean on four row spacings (broadcast, 25, 50 and 100 cm) split by plant population (high and low) in a field infested with the Rhizoctonia Aerial Blight pathogen Rhizoctonia solani AG-1. Duncan's New Multiple Range Test ($P < 0.05$) Bars with the same letter are not significantly different. Burden Research Plantation, Baton Rouge, La., 1984.

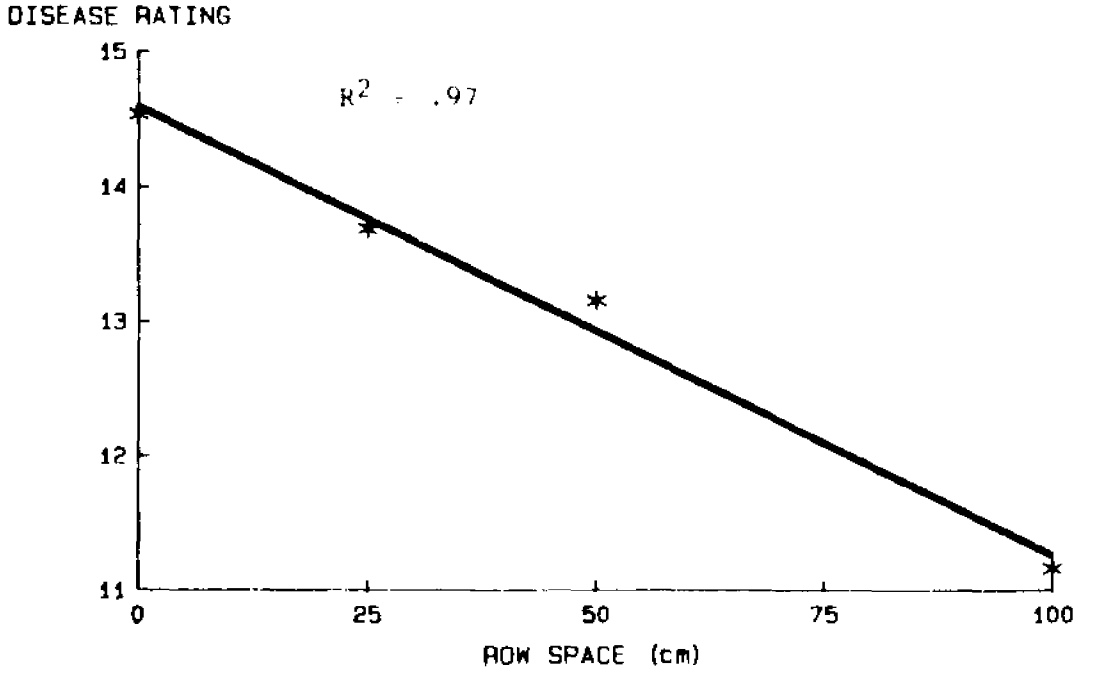


Figure 8. Relationship between Rhizoctonia Aerial Blight disease ratings on 'Davis' soybean and four row spacings (broadcast, 25, 50, and 100 cm). Burden Research Plantation, Baton Rouge, La., 1984.

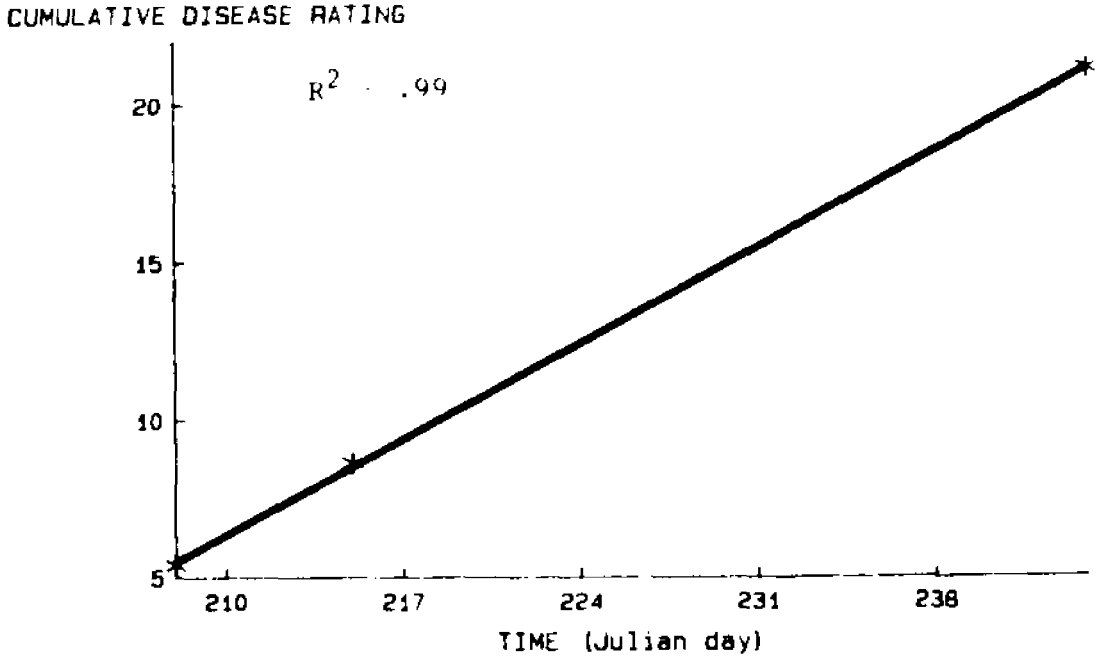


Figure 9. Relationship between Rhizoctonia Aerial Blight disease ratings on 'Davis' soybean and time of season. Burden Research Plantation, Baton Rouge, La., 1984.

Table 7. Influence of row space, plant population, potash, and fungicide treatments on yield of 'Davis' soybean grown in a field infested with the Rhizoctonia Aerial Blight pathogen, Rhizoctonia solani AG-1. Perry, La. 1985.

| Source* | df | Sum of Squares | Mean Squares | F | Prob. |
|---|---|---|---|---|---|
| B | 3 | 69.058 | 23.091 | 1.22 | .3069 |
| R | 2 | 7361.694 | 3680.847 | 194.56 | .0001 |
| P | 1 | 9.513 | 9.513 | 0.50 | .4797 |
| K | 2 | 14.651 | 7.326 | 0.39 | .6798 |
| F | 1 | 0.168 | 0.168 | 0.01 | .9250 |
| RP | 2 | 9.427 | 4.713 | 0.25 | .7799 |
| RK | 4 | 52.202 | 13.050 | 0.69 | .6005 |
| RF | 2 | 16.766 | 8.383 | 0.44 | .6431 |
| PK | 2 | 98.213 | 49.106 | 2.60 | .0790 |
| PF | 1 | 3.176 | 3.176 | 0.17 | .6827 |
| KF | 2 | 55.016 | 27.508 | 1.45 | .2379 |
| Error | 115 | 2175.720 | 18.919 | | |

* B = Block, R = Row Space, P = Plant Population, K = Potassium, F = Fungicide

Table 8. Influence of row space, plant population, potash, and fungicide treatments on Rhizoctonia Aerial Blight disease ratings. Perry, La. 1985.

| Source* | df | Sum of Squares | Mean Squares | F | Prob. |
|---|---|---|---|---|---|
| B | 3 | 55.329 | 18.442 | 1.39 | .2490 |
| R | 2 | 28.827 | 14.413 | 1.09 | .3406 |
| P | 1 | 7.581 | 7.581 | 0.57 | .4511 |
| K | 2 | 20.206 | 10.103 | 0.76 | .4690 |
| F | 1 | 445.576 | 445.576 | 33.60 | .0001 |
| RP | 2 | 46.499 | 23.249 | 1.75 | .1777 |
| RK | 4 | 119.249 | 29.812 | 2.25 | .0681 |
| RF | 2 | 28.049 | 14.024 | 1.06 | .3505 |
| PK | 2 | 71.429 | 35.715 | 2.69 | .0717 |
| PF | 1 | 0.471 | 0.471 | 0.04 | .8508 |
| KF | 2 | 460.044 | 230.022 | 17.35 | .0001 |
| Error | 115 | 1524.485 | 13.256 | | |

* B = Block, R = Row Space, P = Plant Population, K = Potassium, F = Fungicide

Table 9. Variables described by Principal Component Analysis (Proc Factor, SAS) from the Rhizoctonia Aerial Blight disease management study. Perry, La. 1985.

| Components Measured from Tissue Analysis | FACTORS[3] 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Acid Detergent Fiber | 0.15736[1] | 0.06438 | <u>0.86097</u> | -0.02163 | 0.14283 |
| Lignin | -0.13614[2] | -0.20595 | <u>0.76568</u> | 0.04625 | -0.19314 |
| Phosphorus | <u>0.87327</u> | -0.01427 | 0.03267 | -0.21566 | -0.10938 |
| Potassium | 0.05209 | <u>0.74683</u> | -0.01690 | -0.22121 | 0.10591 |
| Calcium | <u>0.64538</u> | <u>-0.53209</u> | 0.02704 | 0.30354 | -0.03576 |
| Magnesium | 0.10573 | 0.04156 | 0.15625 | <u>0.80987</u> | 0.09377 |
| Manganese | 0.37383 | <u>-0.63481</u> | -0.06150 | -0.19730 | 0.38705 |
| Zinc | -0.18913 | 0.10434 | -0.01655 | 0.04158 | <u>0.87947</u> |
| Iron | <u>0.73931</u> | 0.09408 | -0.03100 | 0.16341 | 0.08022 |
| Sulfur | 0.32389 | <u>0.76381</u> | -0.18420 | 0.11243 | 0.05176 |
| Nitrogen | <u>0.74828</u> | 0.17226 | 0.05112 | -0.23998 | -0.27385 |
| Leaf Area | -0.15576 | -0.08866 | -0.11595 | <u>0.63148</u> | -0.05570 |

1. Negative coefficients are inversely proportional to positive coefficients.

2. Underlined coefficients are correlated with each other within a given factor.

3. Factors are orthogonal.

Table 10. Influence of plant nutrients (Factors), row space, fungicide and potash on yield of 'Davis' soybean grown in a field infested with the Rhizoctonia Aerial Blight pathogen, Rhizotonia solani. Perry, La., 1985.

| Source* | df | Sum of Squares | Mean Squares | F | Prob. |
|---|---|---|---|---|---|
| B | 3 | 89.349 | 29.783 | 1.81 | .1493 |
| R | 2 | 6624.006 | 3312.003 | 201.03 | .0001 |
| F | 1 | 3.286 | 3.286 | 0.20 | .6559 |
| K | 2 | 29.236 | 14.618 | 0.89 | .4144 |
| FK | 2 | 126.531 | 63.266 | 3.84 | .0241 |
| FACTOR1 | 1 | 99.021 | 99.021 | 6.01 | .0156 |
| FACTOR2 | 1 | 4.473 | 4.473 | 0.27 | .6033 |
| FACTOR3 | 1 | 20.179 | 20.179 | 1.22 | .2706 |
| FACTOR4 | 1 | 253.544 | 253.544 | 15.39 | .0001 |
| FACTOR5 | 1 | 5.874 | 5.874 | 0.36 | .5516 |

* B = Block, R = Rowspace, F = Fungicide, K = Potassium. FACTOR 1 = the plant nutrients that were correlated are: P, Ca, Fe, and N; FACTOR 2 = the plant nutrients that were correlated are: Ca, Mn, and N; FACTOR 3 = the correlation between acid detergent fiber and lignin; FACTOR 4 = the plant nutrient Mg and Leaf Area Indices were correlated; FACTOR 5 = Zn was the only significant nutrient in this factor.

Table 11. Influence of plant nutrients (Factors), row space, fungicide, and potash on Rhizoctonia Aerial Blight disease ratings on 'Davis' soybean, Perry, La., 1985.

| Source* | df | Sum of Squares | Mean Squares | F | Prob. |
|---|---|---|---|---|---|
| B | 3 | 59.198 | 19.733 | 1.63 | .1848 |
| R | 2 | 41.544 | 20.772 | 1.72 | .1332 |
| F | 1 | 348.496 | 348.496 | 28.87 | .0001 |
| K | 2 | 11.772 | 5.886 | 0.49 | .6153 |
| FK | 2 | 360.838 | 180.418 | 14.95 | .0001 |
| FACTOR1 | 1 | 96.626 | 96.626 | 8.09 | .0052 |
| FACTOR2 | 1 | 6.438 | 6.438 | 0.53 | .4666 |
| FACTOR3 | 1 | 0.154 | 0.154 | 0.01 | .9103 |
| FACTOR4 | 1 | 172.241 | 172.241 | 14.27 | .0002 |
| FACTOR5 | 1 | 38.734 | 38.734 | 3.21 | .0757 |

* B = Block, R = Row Space, F = Fungicide, K = Potassium. FACTOR 1 = the plant nutrients that were correlated are: P, Ca, Fe, and N; FACTOR 2 = the plant nutrients that were correlated are: Ca, Mn, and N; FACTOR 3 = the correlation between acid detergent fiber and lignin; FACTOR 4 = the plant nutrient Mg and leaf area indices were correlated; FACTOR 5 = Zn was the only significant nutrient in this factor.

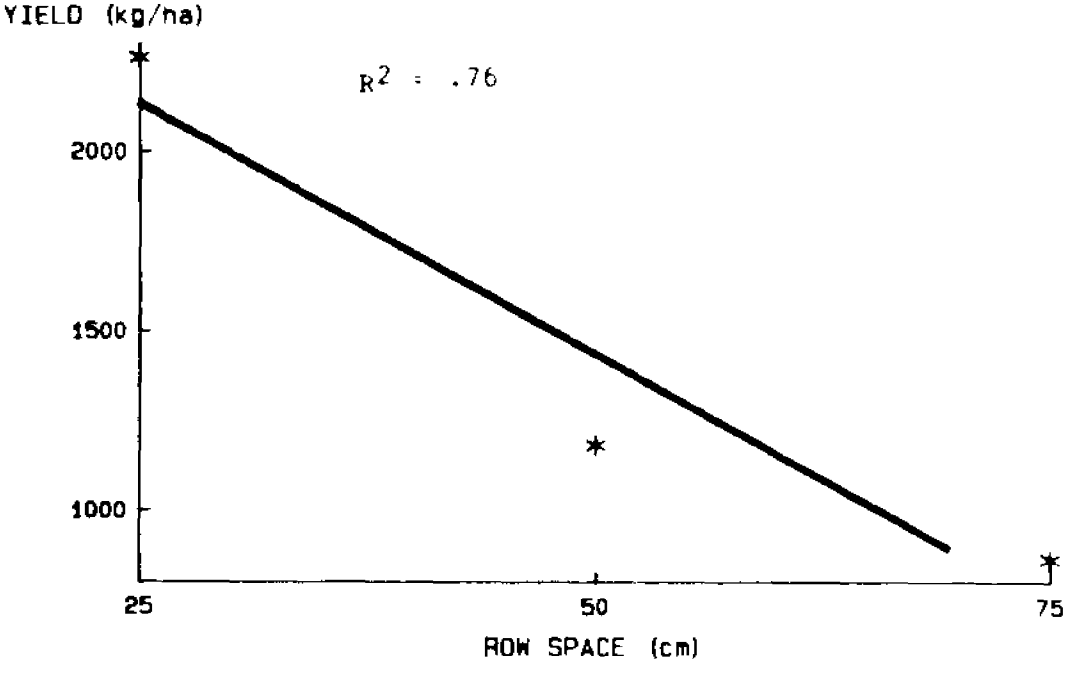


Figure 10. Relationship between 'Davis' soybean yield and three row spacings (25, 50, and 75 cm) in a field infested with the Rhizoctonia Aerial Blight pathogen Rhizoctonia solani AG-1. Perry, La., 1985.

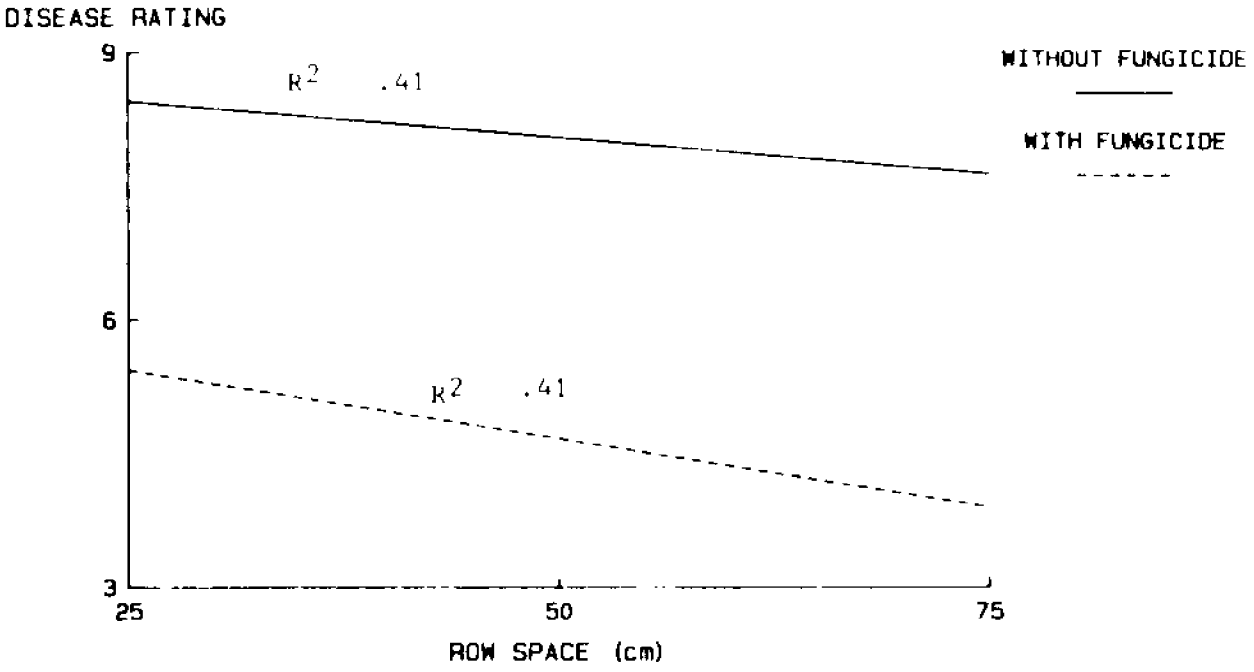


Figure 11. Relationship between Rhizoctonia Aerial Blight disease ratings on 'Davis' soybean and row space (25, 50, and 75 cm) by fungicide (with and without). Perry, La. 1985

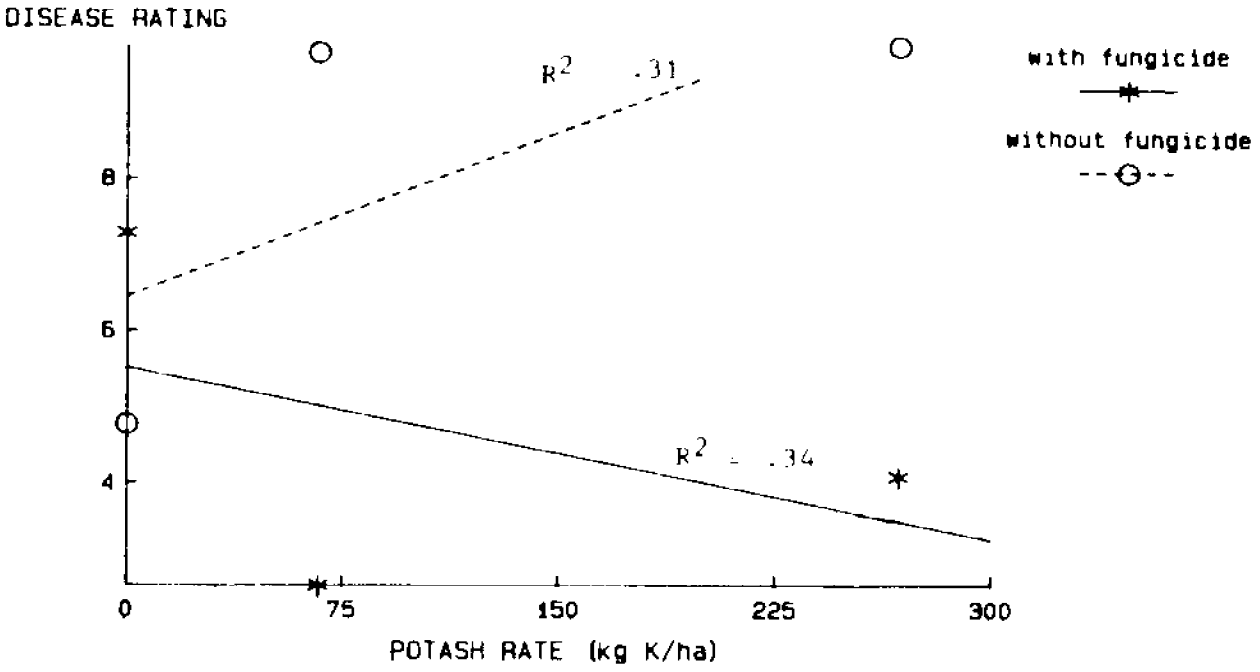


Figure 12. Relationship between Rhizoctonia Aerial Blight disease ratings and potash by fungicide treatments (with and without). Perry, La., 1985.

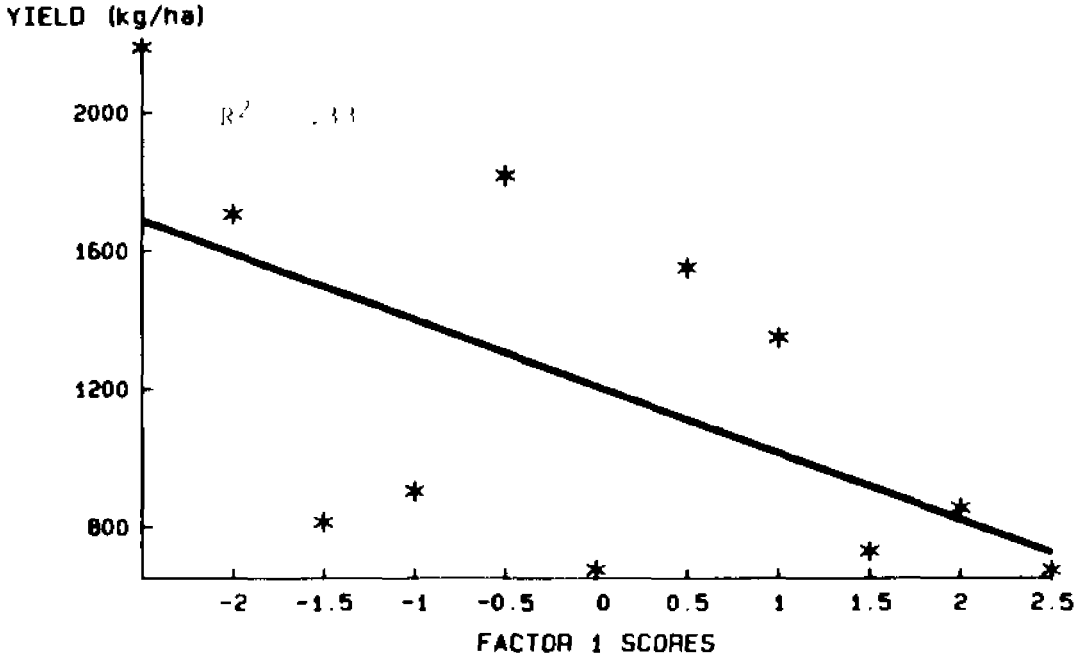


Figure 13. Relationship between 'Davis' soybean yield and Factor 1 (the plant nutrients P, Ca, Fe, and N are correlated and considered as a single variable as determined through factor analysis (6)). Perry, La., 1985.

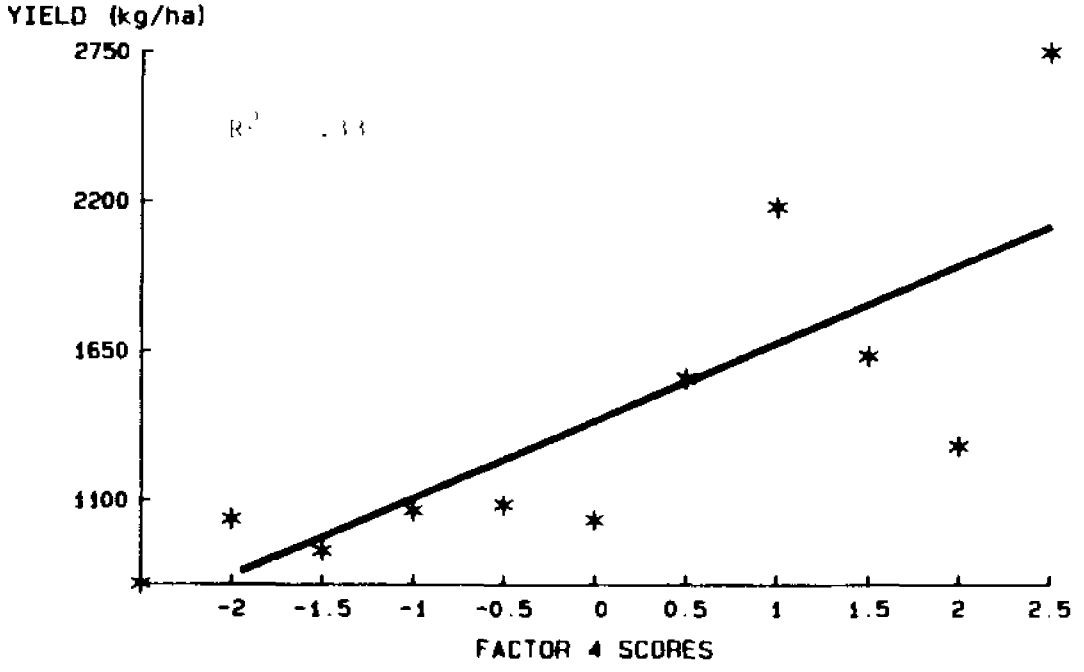


Figure 14. Relationship between 'Davis' Soybean yield and Factor 4 (the plant nutrient Mg and leaf area indices are correlated and considered as a single variable as determined through factor analysis (6)). Perry, La., 1985.

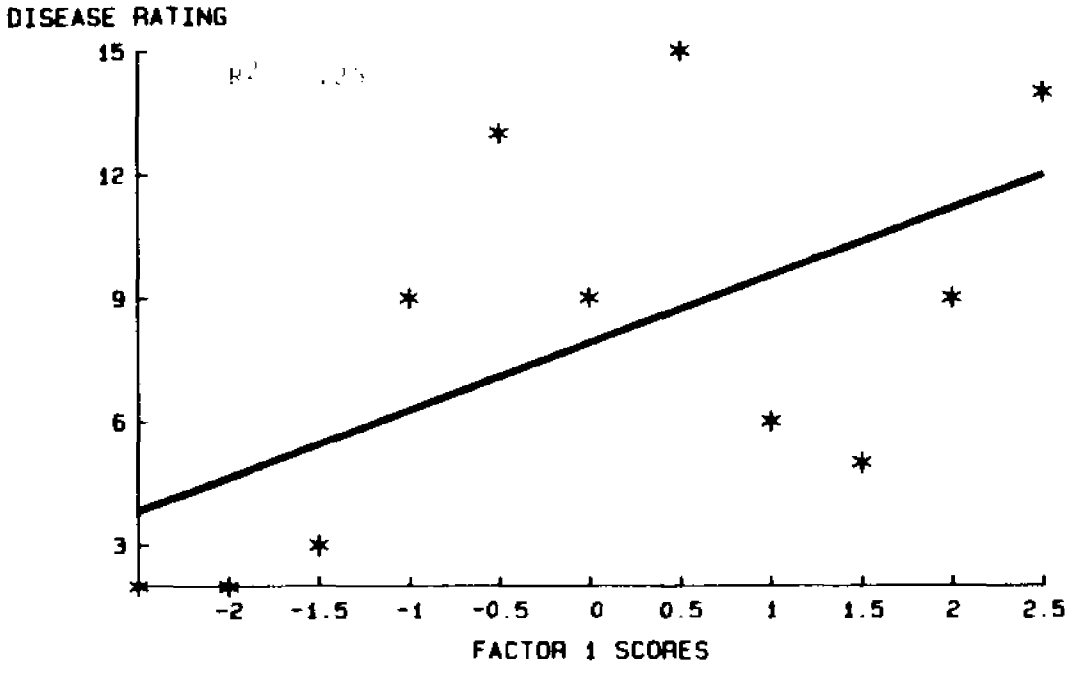


Figure 15. Relationship between Rhizoctonia Aerial Blight disease ratings and Factor 1 (the plant nutrients P, Ca, Fe, and N are correlated and considered as a single variable as determined through factor analysis (6)). Perry, La., 1985.

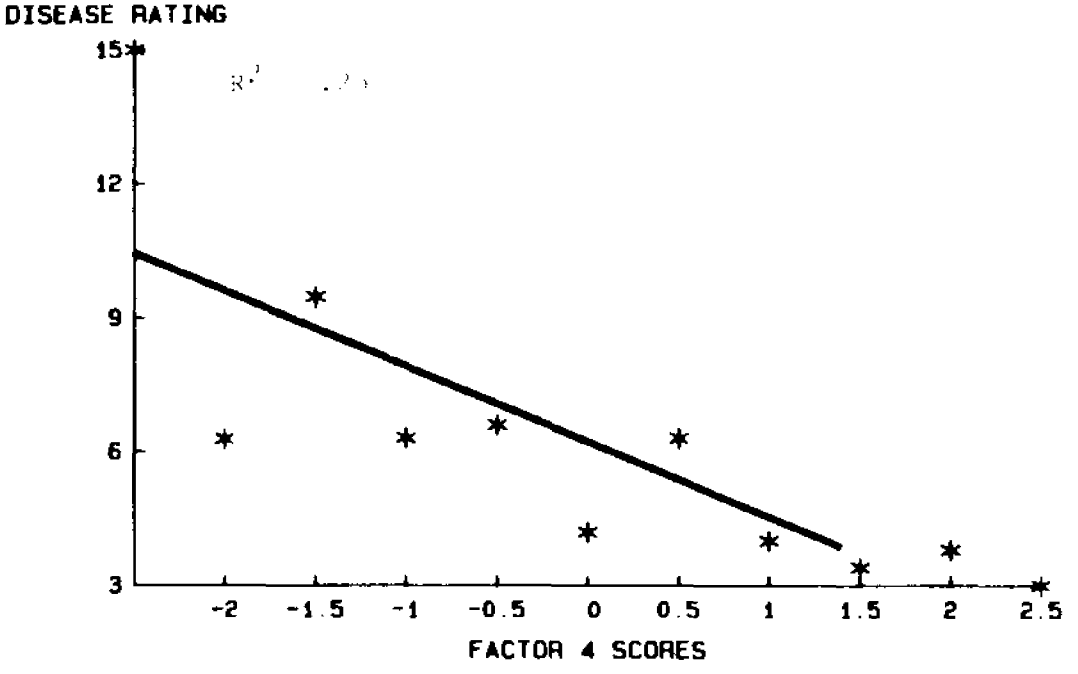


Figure 16. Relationship between Rhizoctonia Aerial Blight disease ratings and Factor 4 (the plant nutrient Mg and leaf area indices are correlated and considered as a single variable as determined through factor analysis (6)). Perry, La., 1985.

## APPENDIX 2

Program for Factor Analysis of 1985 field data for Rhizoctonia Aerial Blight cultural practices study. The Program was run on the Statistical Analysis System (SAS) at Louisiana State University.

```
GARY JOYE PROGRAM -
LIBNAME OUT 'C:SASPROG';
OPTIONS NOCENTER LS = 80 PS=60;
DATA OUT.GARY3; INFILE 'C:\SASPROG\GARY1.DAT';
INPUT B T ADF LIG R D PO (K) F Y D1 D2 LAI P K CA MG MN ZN FE
S N;
PROC SORT; BY B R D PO F;
PROC FACTOR OUT=OUT.GARY4 METHOD=PRIN S C ROTATE=VARIMAX SCREE
EV N=5;
VAR ADF LIG P K CA MG MN ZN FE S N LAI;
PROC PRINT;
PROC GLM;
MODEL Y D1 D2 = FACTOR1 FACTOR2 FACTOR3 FACTOR4 FACTOR5;
PROC GLM; CLASSES B R D F PO;
MODEL Y D2 = B R F|PO FACTOR1 FACTOR2 FACTOR3 FACTOR4 FACTOR5;
RUN;
```

# Chapter III

## Anatomical and Morphological Changes and Survival of Sclerotia of Rhizoctonia solani AG-1 Buried in Soil

### Introduction

Sclerotia are reported to be the primary inoculum of Rhizoctonia solani (4,5,8,10,12,14,19). Diseases caused by Rhizoctonia solani are positively correlated with inoculum density (1,8,15). The survivability of sclerotia in Rhizoctonia species and other soil-borne fungi varies from a few months to several years (15). Previous research on the survival of sclerotia of the RAB fungus in Louisiana is incomplete (7). Fontenot (7) had conducted experiments to determine the survival of sclerotia in soil but his experiments were accidentally destroyed before their completion. The data that he was able to collect suggested that, as time progressed, the percentage of germinating sclerotia was reduced.

Although considerable work has been reported on the growth and development of sclerotia of R. solani (2,3,9,13,16,17,19) none has been published on the anatomical and morphological changes that occur in sclerotia buried in soil. Sclerotia may appear on any above-ground plant parts (12). They are initially small, cottony white spheres which within 36 to 40 h become dark brown and hardened. Sclerotia germination occurs within 24-48 h under optimum environmental conditions (15). Structures commonly associated with

sclerotial cells of Rhizoctonia solani are dolipores in septae, multinucleated monilioid cells, a gelatinous matrix on the exterior of the cell wall, and cells with double walls (2,3,9,13,16,17,19). Reports on sclerotial morphology, however, were made from observation of sclerotia not subjected to environmental stresses. The objective of this research was to determine the length of time sclerotia remain viable in field soil and to examine with scanning and thin-section electron microscopy the morphology of sclerotia collected from various soil depths at varying intervals of time.

## Materials and Methods

### Survival of Sclerotia

#### Preliminary study

The isolate of R. solani (465 AG-1) used in this study was provided by E. E. Butler, University of California, Berkeley, 1984. The organism was cultured on 2% water agar in 9 x 1.5 cm petri dishes. Well-formed sclerotia without clustering were produced in this fashion. Clay pots, 46 cm in diameter at the rim and 50.1 cm deep, were filled with sterilized potting soil (one part each of soil, sand, and peatmoss). Five sclerotia were enclosed within a 100 mesh (.5 mm diameter) nylon screen bag (2 cm ). The bag edges were stapled to prevent loss of sclerotia in the soil. A nylon string approximately 45 cm long was stapled to each bag. A 10 cm wooden label was attached to the opposite end. The bags of sclerotia were placed on the soil surface (0 cm) and at

depths of 5, 10, or 25 cm (1 set). Three sets of sclerotia were buried for each pot giving a total of 72 bags of sclerotia. One set of sclerotia was harvested on six different intervals (8, 16, 24, 32, 40, and 48 wk). All sclerotia were buried on January 20, 1984. Sclerotial bags were removed from the pots with a garden spade. The bags of sclerotia were washed with tap water to remove adhearing soil and surface sterilized in a solution of 10% sodium hypochlorite and 10% ethanol for 2 minutes. After sterilization, bags were then washed in a stream of distilled water for 5 minutes to remove the sterilizing solution. Sclerotia were removed from the nylon bags and placed on 2% water agar in 9 cm x 1.5 cm petri dishes. Germination percent was used as the measure of viability. Positive reidentification of the fungus was determined by light microscopy.

Field study

A similar experiment was conducted under field conditions. The isolate of Rhizoctonia solani for this experiment was obtained from a cultivar 'Davis' soybean plant in a field near Perry, La. Sclerotia were produced and buried in a garden plot on the LSU campus, Baton Rouge, La. as previously described. The soil texture was classed as a heavy clay and had been planted in 'Davis' soybeans the previous year. Sets (as described above) were replicated ten times. Sclerotia were buried on February 16, 1985. Sclerotia were collected at 8 to 10 week intervals. Sclerotia were harvested, surface sterilized, and rinsed by the same procedure

previously described. Sclerotial viability was determined as percent germination. Data was subjected to analysis of variance procedures and regression analysis. The sclerotia harvested in this study were stored at 4 C for scanning and thin-section electron microscopy study.

Fixation for Scanning Electron Microscopy and Thin-Section Electron Microscopy

Sclerotia recovered from the field survival study that were to be examined with scanning electron microscope were fixed for 5 h in a solution of 6% gluteraldehyde, 1% osmium tetroxide, and .1 M cacodylate buffer at pH 7.0. The sclerotia were dehydrated through an ethanol series (50% ethanol for 10 minutes, 72% ethanol for 10 minutes, 85% ethanol for 10 minutes, 95% ethanol for 10 minutes and 3 ten minute intervals at 100% ethanol), critical point dried with a Denten Vacuum critical point bomb (Denten Vacuum, Inc., Cherry Hill, New Jersey), mounted on observation studs, and coated with 200 A of platinum-gold in a Sputter Coater (Hummer Technics, Alexandria, Virginia)(18). A Hitachi model S-500 scanning electron microscope (Hitachi, LTD, Tokyo, Japan) was used for viewing.

Sclerotia used for thin-section electron micropscopy were fixed for 5 days in a solution of 1% acrolein, 2% gluteraldehyde and .1 M cacodylate buffer at ph 7.0. Sclerotia were rinsed in buffer for 24 h and stained for 24 h in 4% osmium tetroxide. Fixed sclerotia were dehydrated through an ethanol series as described above and embedded with L. R. White embedding material (Ernest F. Fullman,

Inc., Latham, New York) (18). Thin-sections were made by using a Sorvall, Porter/Blum Ulta-microtome, model MT-2 (Dupont Instruments, Newtown, Connecticut) fitted with a glass knife made from a 6.4 mm thick and 2 cm square piece of glass using a LKB Knifemaker, model 7801-A (LKB-Produktur AB, Stockholm, Sweden). The thin sections were stained with 2% lead citrate for 1 minute and carbon coated using a Mikros Vacuum Evaporator, model VE10 (Mikros, Inc., Portland, Oregon). Twenty immature sclerotia (less than 30 hours old) and twenty mature (greater than 30 hours old) sclerotia collected from all soil levels at both 50 and 384 days were examined. A JEOL model 100-CX transmission electron microscope (JEOL, USA, Inc., Peabody, Massachusetts) was used for thin-section electron microscopy.

Tables and Figures of analysis of data and electron micrographs are in the appendix following the literature cited.

## Results and Discussion

### Survival of Sclerotia

In both the preliminary greenhouse and field experiments survival of sclerotia was influenced by time (Tables 12 and 13). Survival was reduced by an average of 60 and 70 % for all soil levels over a 48 and 55 wk period respectively (Figures 17 & 18). Viability of sclerotia was variable for all soil levels but was not significantly different from sclerotia placed on the soil surface (0 cm). The variability in the survival of sclerotia was consistent

with previous experiments on the longevity of other isolates of R. solani (15). In general, longevity studies of sclerotia have taken place in vitro with sclerotia exposed to different temperature, light, and moisture regimes. From those studies, several generalizations were suggested. Survival was favored by low temperatures (5 C) (15). Resistance to high temperature (> 45 C) is greater under dry than moist conditions (15). Under severe stress (15), the number of viable sclerotia decrease progressively with time.

Townsend and Willet (16) consider the dense cellular content, pigmentation, and nearly impermeable wall of the sclerotial cells as responsible for resistance to high temperature. Matz (11) believed that within aerial strains, the sclerotia are more distinct and of a harder consistancy than root strains. This may be an adaptation to the aerial habitat.

## Observations on Anatomical and Morphological Changes in Sclerotia Buried in Soil

### Scanning Electron Microscopy

The principal structure of sclerotia of R. solani has been reported to be composed of a mass of monilioid cells (9) while other investigators have indicated that sclerotia of isolates of R. solani were composed entirely of undifferentiated hyphae (3). Examination by scanning electron microscopy of the exterior of sclerotia collected from 'Davis' soybean leaves showed that the structures were composed of a mass of undifferentiated mycelial

hyphae aggregated into a sphere-like structure (Figure 19). Upon cross-sectioning sclerotia, three distinct regions were discernable which have been designated the rind, the medulla, and the pith (Figure 20). The rind is an outer layer of elongated mycelial hyphal cells, loosely bound around the medulla, which becomes empty with age. The rind of the sclerotia of other isolates of R. solani have been described in a similar manner (3). The medulla is the area below and adjacent to the rind. It is composed of densely compacted monilioid cells. With time, many cells of the medulla were also observed to become empty. The pith, the third and innermost region, is composed of a mass of loosely bound monilioid cells. The medulla and the pith may be distinguished by a gradual change in the cell density. The region between the rind and the medulla is shown in Figure 21. There are no other reports distinguishing three regions of sclerotial structure. Nonaka and Kaku (13) have indicated that there was no differentiation between an inner and outer layer of the sclerotia of an isolate of R. solani known to cause Rice Sheath Blight. However, Hashiba and Mogi (9) concluded that the sclerotia of the Rice Sheath Blight fungus could be differentiated into two well-defined structures, an inner layer of livings cells and an outer layer of empty cells. The outer layer was described as loosely bound hyphal cells which become empty with age. The inner layer of living cells was described as a loose mass of monilioid cells. Tu and Kimbrough (17) have indicated that mature sclerotia of species of the Rhizoctonia

complex lack well-defined structural zones.

Thin-section Electron Microscopy

There were no anatomical or morphological differences, at any time, between sclerotia at soil depths of 5, 10, and 25 cm or when compared to sclerotia on the soil surface (0 cm). The immature sclerotia (less than 30 h old and never placed in soil) were usually in a state of rapid growth. The monilioid cells of immature sclerotia have thin cell walls (0.1 μm in width), incomplete septal formation, large evacuated areas within the cells, and easily distinguishable cellular organizaton (Figure 22). The presence of the dolipore (septal pore apparatus) (17) was evidence that cytoplasmic exchange occurs between adjacent cells. This is a characteristic structure of the Basidiomycetes (17). The thick and thin areas of the cell walls (Figure 22) of immature sclerotial cells may be due to inconsistent staining of the cell wall, and/or variations in the section thicknesses. These areas may actually be significant modifications with a specific function during periods of rapid growth. These areas of the cell walls have not been previously reported. The dolipores in these sclerotia were 1 to 3 μm in diameter. Tu and Kimbrough (17) have reported dolipores averaging 2μm in diameter and were frequently observed in other isolates of R. solani. Invaginations of the cell walls in many cells were also observed (Figure 23). The invaginations have been reported to be the initial formation of septa or the production of monilioid cells (2). The formation of cross walls by invagination is reported to

take approximately 10 minutes in young hyphae (2).

Mature sclerotia were divided into two groups; those harvested 50 days (50 d) after burial and those harvested at 384 days (384 d) after burial. Sclerotia harvested at 50 d showed cellular modifications not present in the immature sclerotia. The most striking change observed was that the medulla was composed of both non-evacuated and evacuated monilioid cells. With the exception of electron dense structures, which appeared to be randomly scattered throughout the protoplasm, the cellular organization was not discernible in sclerotia buried for 50 d (Figure 24). The electron dense structures closely resemble glycogen granules described by Fawsett (6). Glycogen granules stain darkly when treated with osmium tetroxide and are 'rosette' in form.

Viable (non-evacuated cells) and non-viable cells (evacuated cells) had thickened cell walls (0.5 μm). Bracker and Butler (2) indicated that cell wall thickening was due to layering of the lamellae and was formed by centripetal deposition. Dark striations (Figure 25) are visible in the cell wall which indicate that layering of the lamellae has occurred. The function of thickening of cells walls has not been examined but since sclerotia are survival propagules the explanation may be that of protection from the environment. Also, there was evidence that actively growing cells penetrated other empty monilioid cells (Figure 25). Butler and Bracker (3) reported 'cell within cell formation' in sclerotia of old colonies (26 wk) of R. solani and suggested that these

formations may be cells which had formed a secondary wall or that adjacent cells had grown and divided into neighboring cells. Within the medulla and pith layers, some cells appeared to have double walls (Figure 26). The formation of cells with double walls has been reported in hyphae (2). Since the monilioid cell arose from hyphal cells the occurrence of double walled cells in sclerotia would not be unreasonable.

In sclerotia buried for 384 d, no signifcant structural modifications had occurred except that there was a greater amount of cytoplasmic evacuation in the monilioid cells, as it was only a rare occasion that viable cells were located in sectioned sclerotia. The most pronounced change other than the loss of viable cytoplasm was the increase in cell wall thickness (Figure 27). Cell walls in some areas were more than 1.0 μm in width and varied from less than 0.5 μm to more than a 1.0 μm within the same cell wall. In sclerotia of other isolates of R. solani, variation in cell wall thickness has been reported to occur in pure culture (3,17). Deteriorated dolipores were commonly found in these sclerotia (Figure 28). In living cells adjacent to empty cells, the dolipore is known to form a plug in the passage to prevent cytoplasm from escaping from the living cell (3). The cytoplasm of the 384 d sclerotia was similar in appearance as the 50 d sclerotia. Given that the sclerotia were probably in a state of low activity and that the cytoplasm of the cells was darkly stained, specific organelles of the cytoplasm could not be distinguished. As indicated above, electron dense structures distributed throughout

the cytoplasm were thought to be glycogen granules (Figure 29).

## Conclusions

The following conclusions are drawn:

1. The viability of sclerotia of the Rhizoctonia solani isolate which caused aerial blight of soybean in Louisiana was reduced significantly over time when they were on the soil surface or buried in soil.

2. The sclerotia of the R. solani isolate used in this study which causes RAB in Louisiana consist of three distinct internal regions: the rind, the medulla and the pith. The rind is composed of typical elongated hyphal cells. The medulla is composed of a dense mass of monilioid cells, and the pith is a loosely formed mass of monilioid cells. The medulla and pith are separated by cellular gradations between each region.

3. The anatomical and morphological changes in buried sclerotia were similar to those described previously in the literature. Cells of immature sclerotia are in a state of rapid growth and division. Cells of immature sclerotia may have large evacuated areas within the cytoplasm. The cell wall may be less than .5μm thick. The dolipore is frequently intact within the septae. Also, there is frequently incomplete septal formation. Sclerotia that had been buried 50 d were observed to be in a state of dormancy given to reduced cytoplasmic activity and abundance of what are thought to be glycogen particles. There is less viable cellular material in these sclerotia. Structures such as double walls and 'cell within

cell formation' were observed. Cell wall thickening was observed as layering of the lamellae within the cell walls. These structural changes were thought to aid in the survival of sclerotia. Sclerotia that were buried for greater than a one year period (384 d) had the smallest percentage of viability of all sclerotia examined. Observation of non-evacuated cellular material in sclerotia buried for more than a year was a rare occurence. Both the non-evacuated and evacuated cells that were observed had thickened cell walls that varied from .5 to > 1 μm .

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# APPENDIX

# OF

# TABLES AND FIGURES

Table 12. Influence of soil depth and time on the survival of sclerotia of Rhizoctonia solani in sterile soil. A preliminary experiment.

| Source* | df | Sum of Squares | Mean Squares | F | Prob. |
|---|---|---|---|---|---|
| Rep | 2 | 700.000 | 350.000 | 0.95 | .3932 |
| Da | 5 | 16466.667 | 3293.200 | 8.96 | .0001 |
| De | 3 | 111.111 | 37.037 | 0.10 | .9591 |
| DaDe | 15 | 3222.222 | 214.815 | 0.58 | .9714 |
| Error | 46 | 20500.000 | 820.000 | | |

* Rep = Replications, Da = Day of collection, De = Soil Depth.

Table 13. Influence of soil depth and time on the survival of sclerotia of Rhizoctonia solani in soil under field conditions.

| Source* | df | Sum of Squares | Mean Squares | F | Prob. |
|---|---|---|---|---|---|
| Rep | 9 | 17075.000 | 1897.222 | 2.39 | .0146 |
| Da | 5 | 77855.000 | 15571.000 | 19.58 | .0001 |
| De | 3 | 7658.333 | 2552.777 | 3.21 | .0246 |
| RepDa | 45 | 33795.000 | 751.000 | 0.94 | .5769 |
| Error | 162 | 128850.000 | 795.370 | | |

Rep = Replications, Da = Day of collection, De = Soil Depth

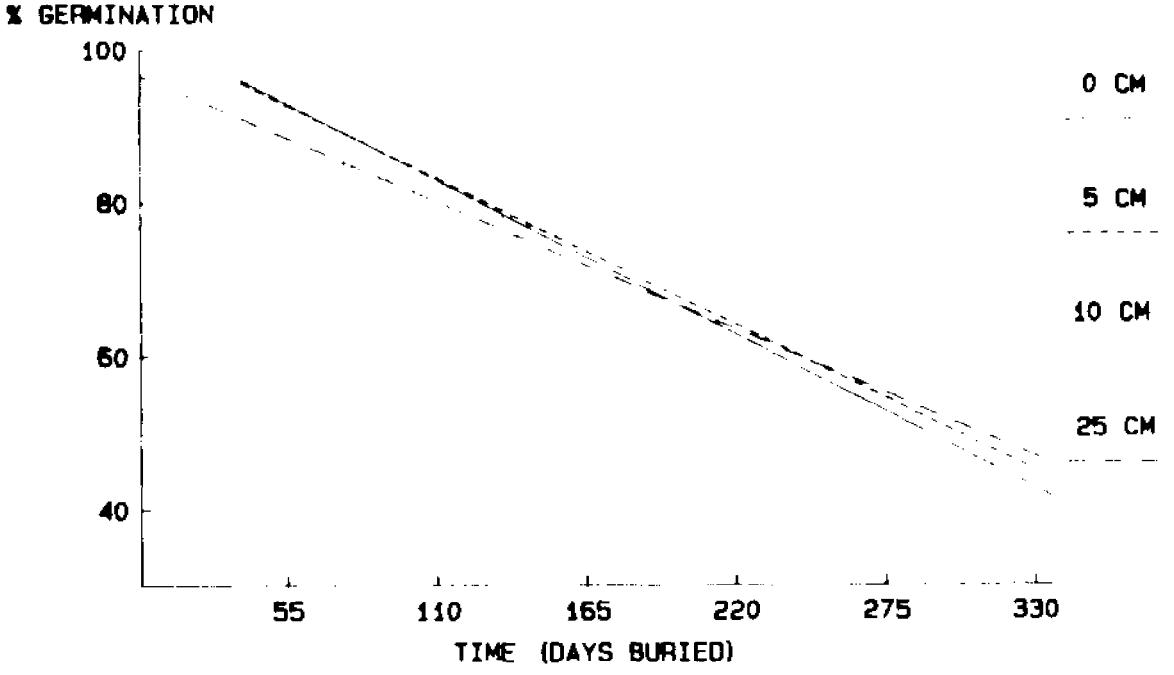


Figure 17. Relationship between survival (% germination) of sclerotia of Rhizoctonai solani and time buried in sterile soil. $R^2$ = .63, .62, .32, and .35 for 0, 5, 10, and 25 cm soil depths.

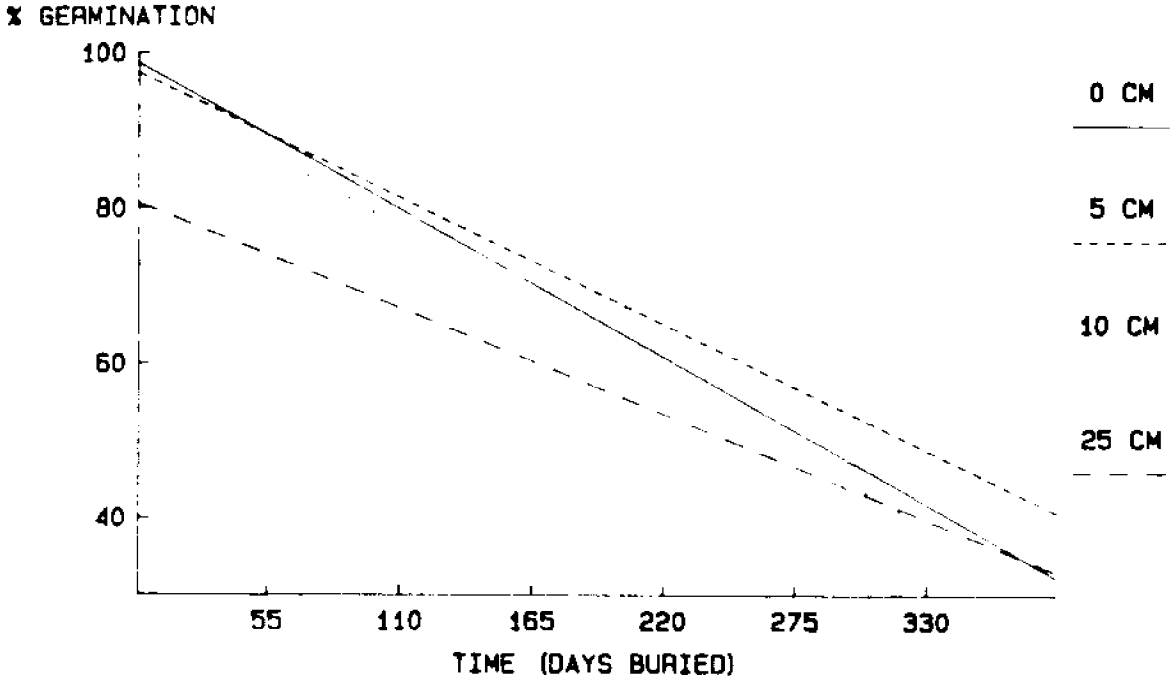


Figure 18. Relationship between survival (% germination) of sclerotia of Rhizoctonia solani and time buried in soil under field conditions. $R^2$ = .50, .45, .46, and .35 for 0, 5, 10, and 25 cm soil depths.

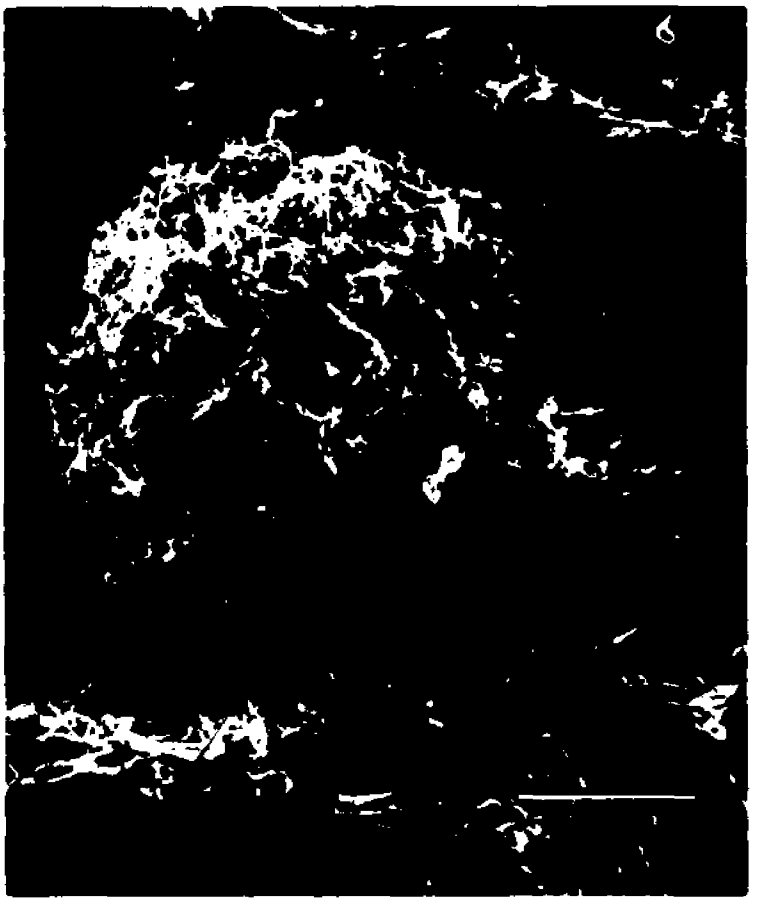

Figure 19. Scanning electron micrograph. A mature (> 14 days) sclerotium of Rhizoctonia solani on a 'Davis' soybean leaf; Magnification = x 95).

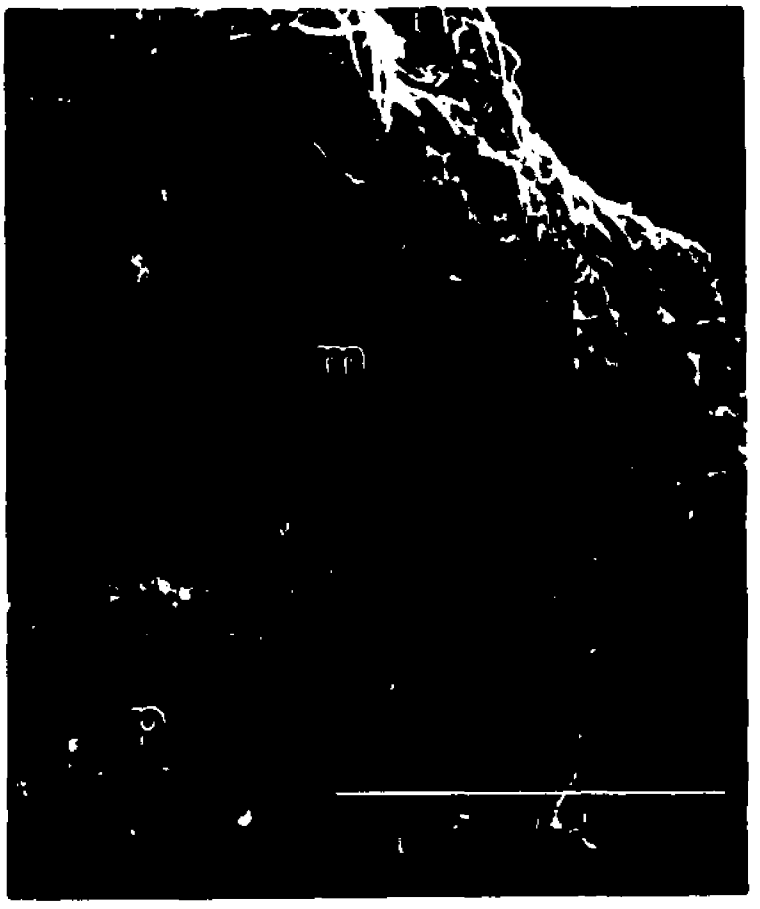

Figure 20. Scanning electron micrograph. A x-section through a sclerotium of Rhizoctonia solani. r, rind; m, medulla; and p, pith. Magnification = x 650.


Figure 21. Scanning electron micrograph. The region between the rind and medulla of a mature (> 14 days old) sclerotium of Rhizoctonia solani. (M) Monilioid cells of medulla and (R) hyphal cells of rind. Magnification = x 1050.


Figure 22. Thin-section electron micrograph. The monilioid cells of the medulla of an immature (< 30 hours) sclerotium of Rhizoctonia solani. EA, evacuated area; D, dolipore; IS, incomplete septal formation. The arrows indicate variation in electron dense areas (thick and thin areas) of the monilioid cell walls. Magnification = x 5000.


Figure 23. Thin-section electron micrograph. The invagination of a cell wall of a monilioid cell in the medulla of an immature (< 30 hours) sclerotium of Rhizoctonia solani. Magnification = x 16000.

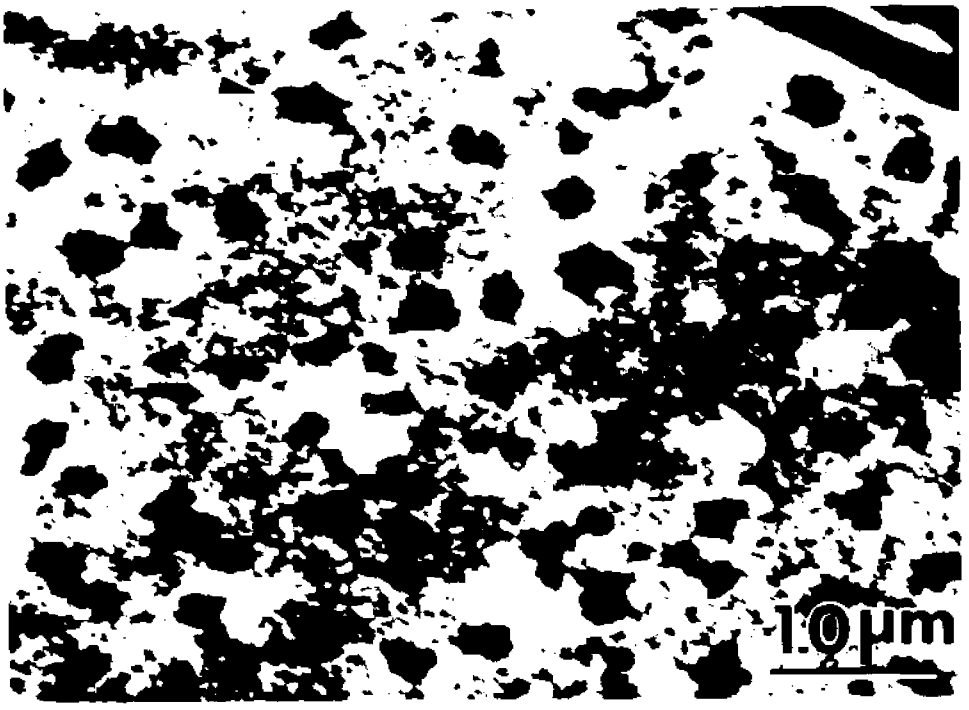


Figure 24. Thin-section electron micrograph. The cytoplasm of a monilioid cell of a sclerotium of Rhizoctonia solani collected from soil after being buried for 50 days. The arrow indicates electron dense structures scattered throughout the cytoplasm. Magnification = x 16000.

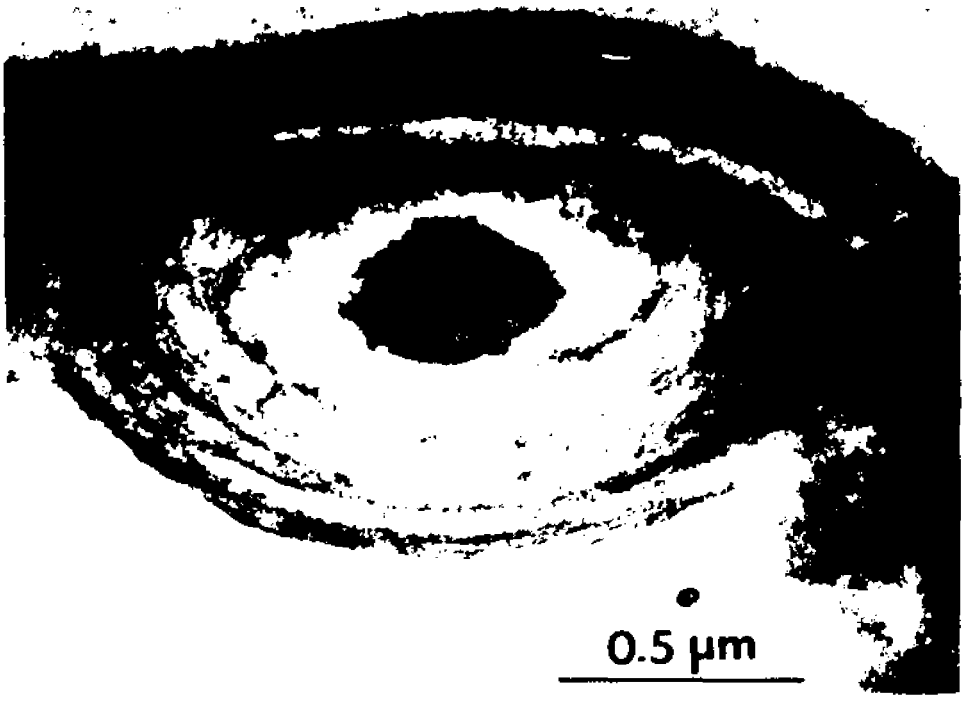


Figure 25. Thin-section electron micrograph. A viable cell has penetrated an empty cell in the medulla of a sclerotium of Rhizoctonia solani which had been buried in soil for 50 days. The arrow indicates layering of the lamellae. Magnification = x 26000.

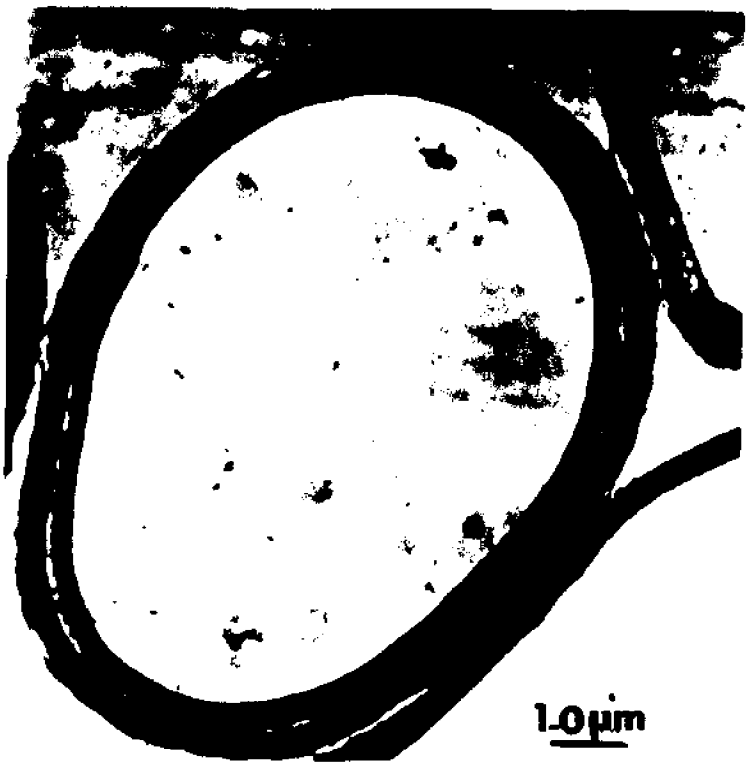


Figure 26. Thin-section electron micrograph. A double walled, evacuated monilioid cell of the medulla of a sclerotium of Rhizoctonia solani which had been buried in soil for 50 days. Magnification = x 5000.

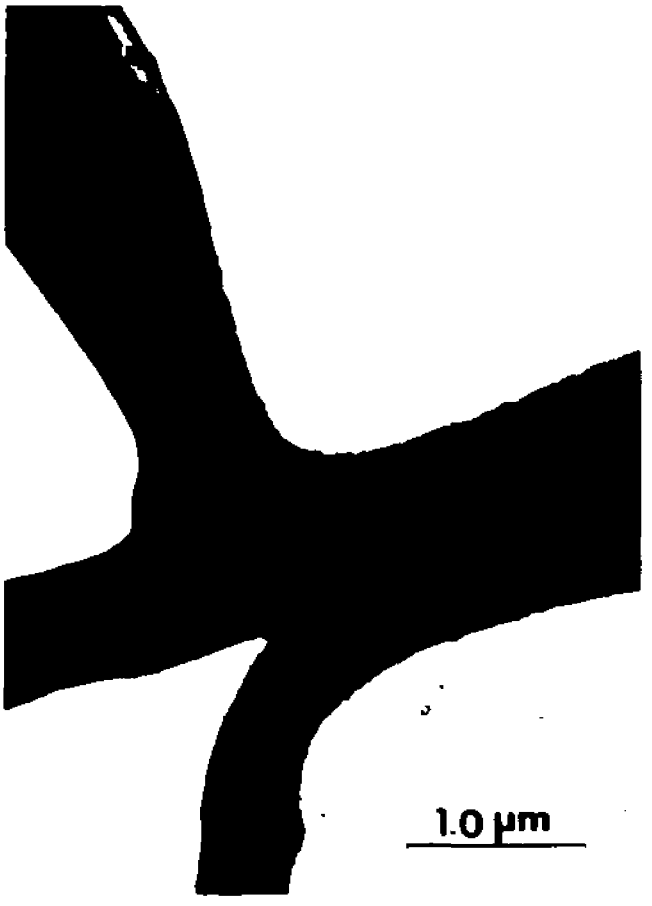


Figure 27. Thin-section electron micrograph. The cell walls of empty monilioid cells of a sclerotium of Rhizoctionia solani which had been buried in soil for 384 days. Magnification = x 66000.

Figure 28. Thin-section electron micrograph. A deteriorated dolipore in a septum of a monilioid cell of a sclerotium of Rhizoctionia solani which had been buried in soil for 384 days. Magnification = x 66000.

Figure 29. Thin-section electron micrograph. The cytoplasm of of a monilioid cell of a sclerotium of Rhizoctonia solani which had been buried in soil for 384 days. The arrow indicates electron dense structures scattered throughout the cytoplasm. Magnification = x 20000.

# VITA

The author, Gary Fischer Joye, was born in Valdosta, Georgia on October 5, 1954. In 1958, his family moved to Thibodaux, Louisiana. In 1963, the family moved to Slidell,La. Gary graduated from Salmen High School in Slidell in 1973. Between working in various occupations (painter, offshore roustabout, carpenter, garment factory worker, seafood cook, disc jockey and occasional farm hand) and four universities (Nicholls State University (Fine arts major), Southeastern Louisiana University (Fine arts major and switched to plant science), Northeast Louisiana University (plant science major), University of Georgia (agronomy major)), he received a Bachelor of Science degree with a major in Agronomy from the University of Georgia in 1980. He received his Master of Science degree with a major in Botany from Northeast Louisiana State University (NLU) in 1982. During his time at NLU he was employed with the Louisiana Agricultural Research Corporation in Monroe and remained there until the fall of 1982. In January 1983, he enrolled at the Louisiana State University to pursue a Ph.D degree in Plant Health.

While a student at LSU, the author married Marianne LaCour on October 1, 1983 and has two children; Dorian and Ciara Nichole.

**DOCTORAL EXAMINATION AND DISSERTATION REPORT**

**Candidate:** Gary F. Joye

**Major Field:** Plant Health

Title of Dissertation: Management of Rhizoctonia Aerial Blight of Soybean and Biology of Sclerotia of Rhizoctonia Solani Kuhn

Approved:

Gerard T. Berggren
Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE

Darrell E. Mutchmor

David J. Boethel

Warren A. Kendrick

Edward C. Mitchell

G. P. Dively

A. K. Macpherson

Date of Examination:

January 22, 1987